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Nutritional Evaluation of Pastures for Dairy Cattle in Louisiana Using Invitro Methods.

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NUTRITIONAL EVALUATION OF PASTURES FOR DAIRY CATTLE IN LOUISIANA USING *IN VITRO* METHODS

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ABSTRACT

This study was conducted to determine the nutritive value of permanent pastures for dairy cattle by means of the artificial rumen technique. Chemical analyses and quality score of pastures were made as additional measures of pasture quality. Climatic and milk production data were obtained to study their interrelationships with the measures of pasture quality.

Permanent pastures grazed as the sole source of roughage by the dairy herd at Iberia Livestock Station, Jeanerette, Louisiana, were used to collect weekly hand-plucked and esophageal-fistula forage samples from June 1 to September 28, 1960. Ten selected cows of different breeds and at 40 to 120 days of their lactation, were used to obtain milk production data.

The artificial rumen was an all-glass system and a washed cell suspension technique was employed. In vitro cellulose digestibility and volatile fatty acid (VFA) production were the criteria used to measure the nutritive value of the forage.

Preliminary studies with the artificial rumen showed that a buffer medium was more desirable than a nutrient medium in forage digestion work. Two-tenth gram forage substrate gave optimal cellulose digestibility. The initial pH of the medium at 6.9 to 7.1

provided optimal microbial activity. Rate of digestion studies showed maximum digestion of the forages at 40 to 48 hours of incubation. Rumen inocula from two steers under different feeding regimes gave similar trends in the in vitro cellulose digestibility of esophageal-fistula pasture samples.

Data from the in vitro forage digestion trials showed that the in vitro cellulose digestibility and VFA production, especially that of acetic acid, of the hand-plucked pasture samples increased when the quality of the pastures tended to be better as indicated by the chemical composition of hand-plucked samples. Correlation analyses indicated significant relationships between the in vitro cellulose digestibility and crude protein, crude fat, calcium, phosphorus, and potassium contents for the hand-plucked pasture samples, and the crude protein and calcium contents for the esophageal-fistula pasture samples. Significantly negative relationships existed between the in vitro cellulose digestibility and crude fiber and nitrogen-free-extract contents for hand-plucked pasture samples. Significant relationships existed between the acetate/propionate ratio produced in vitro and the phosphorus, potassium, and calcium contents of the hand-plucked pasture samples. Pasture quality score was significantly correlated with Y, the difference between actual and expected milk production, although the r^2 value was about six per cent.

Pasture quality score showed a significantly negative correlation with ambient air temperature. Multiple regression analyses indicated a failure of all measures of pasture quality and climate in predicting milk production significantly. R^2 values averaged about 10 per cent. Factors that might be involved in the lactation response of the cows were discussed.

Correlation analyses indicated that the crude protein, crude fiber, potassium, in vitro cellulose digestibility, and acetate/propionate ratio determined from VFA production in vitro, of esophageal-fistula pasture samples were significantly related to those of hand-plucked samples. The esophageal-fistula samples were usually higher in crude fiber, ash, calcium, phosphorus, and sodium and were lower in crude fat and NFE than the hand-plucked samples. Evidence was presented on the possible use of esophageal-fistula forage samples in artificial rumen digestion trials for the nutritional evaluation of pastures.

It was suggested that the artificial rumen procedure used in this study was effective in determining relative weekly trends in the in vitro cellulose digestibility and VFA production of pastures by rumen microorganisms. The artificial rumen procedure appeared to be an efficient and quick method of determining the relative nutritive value of pastures for dairy cattle throughout a grazing season.

I. INTRODUCTION

Pasture forages are the most important feed for dairy cattle in Louisiana. A wide variety of forages can be grown throughout the year because of the sub-tropical climate and relatively mild winters. This will permit a constant source of forage in the form of permanent and supplementary pastures, and also harvested forage in the form of hay, silage, or soilage.

There is a need to study variations in certain plant fractions of pastures as related to the nutrition of the animal. There is evidence that the chemical composition and digestibility of nutrients of pastures fluctuate in a matter of days and at different stages of growth of the pasture plants (12, 13, 61).

During the mid-summer months, there is a marked characteristic decrease in milk yield of dairy herds in Louisiana. It is not known whether this decrease in milk yield is mainly due to the decline in pasture nutritional quality during the summer, or whether it is due to the effect of climate and other external factors on the physiological activity and milk-producing capacity of dairy cows. Johnson and Southwell (52) at the Georgia Coastal Plain Station showed evidence of the influence of weather conditions during the summer on milk production and feed consumption of dairy cows. They

stated that climate had a greater influence on feed consumption than on milk production. Johnston (53) considered that the principal cause of the "summer slump" in milk production is probably due to a decline in quantity and quality of available herbage, probably magnified by poor management. He stated that high body temperatures alone do not necessarily result in lowered milk production if the animals are fed adequate total digestible nutrients (TDN) and their appetites remain normal. Information is thus needed on the extent of the direct (on the animal) and indirect (on pastures and other feedstuffs) effects of climate and other environmental factors during the summer.

Investigation on the indirect effects of climate and adverse environmental factors in the summer would require an accurate and efficient method of determining the nutritive value of pastures for dairy cattle. This has been the goal of research workers for many years. In Louisiana very little work has been done on the nutritional evaluation of pastures. Recently, Bertrand et al. (13) determined the digestibility of permanent pastures for dairy cattle at three different periods in the summer. They reported significant changes in digestibility of nutrients from one period to the next. Their regression analyses indicated that pasture quality score was the only significant variable in predicting milk production. These investigators recommended further research in this area.

Thus accurate and efficient methods of nutritionally evaluating

pastures for dairy cattle in Louisiana need to be investigated. The artificial rumen technique of determining digestibility of forages has been proven to be a fast and reliable method (9, 10, 24, 47, 66). It is less laborious and less expensive than the conventional method of determining forage digestibility (16). It may also prove to be more accurate and precise than other methods of pasture evaluation since the experiments are carried out under controlled conditions in the laboratory (15, 16, 17).

Therefore, this study was designed to employ the artificial rumen technique for pasture evaluation with the following objectives in mind: (a) to determine weekly changes in chemical composition, and the in vitro cellulose digestibility and volatile fatty acid (VFA) production from permanent pastures; (b) to study the interrelationships of the weekly changes in chemical composition, in vitro digestibility determinations, and quality score of the pastures, and also the climatic and milk production data; and (c) to evaluate the esophageal-fistula method of collecting pasture samples.

II. REVIEW OF LITERATURE

A. The Use and Economic Importance of Pastures for Dairy Cattle

Pasture is the natural feed for dairy cattle and in many respects the best. Abundance of good pasture provides most of the requirements of a good dairy ration, especially that of energy, for economical milk production (19, 49, 64).

Thirty-five per cent of the total feed for livestock in the United States is in the form of pasture (78). Except in specialized dairies in a few small sections of the United States, pasture is of the greatest importance in the production of milk (46). Pastures offer the livestock producer his cheapest and most economic source of feed (3). According to Dow (34), when cows were pastured from mid-May to mid-October at the Maine Station, there was a reduction of 46 per cent in the amount of grain fed, 86 per cent in roughage fed other than pasture, and 31 per cent less labor used daily in caring for the cows. In addition, studies in New Jersey showed that pasture was the cheapest source of total digestible nutrients (TDN) for cattle (19). The economy of pasture is well shown in studies conducted by the United States Department of Agriculture in seven dairy districts of this country (62). In these areas pasturage furnished nearly one-third of the total

nutrients consumed by the cows during the year, but the cost of the pasturage was only one-seventh of the total animal feed cost. The effectiveness of pasture in reducing the cost of milk production was shown by studies on farms in various areas of New York. The average total cost of producing milk on these farms during the grazing season was only 55 per cent as great per 100 pounds of milk as it was during the winter barn-feeding season (62).

Permanent pasture is one of the most economical feeds available for dairy cattle especially in the southern United States, where pastures provide the longest grazing period throughout the year (78). Hardison (43) of the Virginia station stated that over 75 per cent of the feed consumed by ruminants comes from forages which is the most important source of energy.

Aside from being an important source of energy, mixed legume pastures provide a good source of protein, minerals, and vitamins (62). Green pastures are especially rich in carotene, an important source of vitamin A for dairy cattle.

B. Methods of Sampling Forage

1. Effect of Animal Grazing Behavior

The technique developed by Reid and associates (68, 69) for estimating herbage intake and digestibility by grazing animals, has made possible the study of the effect and degree of selective grazing

by cattle. "Selective grazing" was defined as that in which animals select a diet of a chemical composition different from that of the whole, clipped herbage. Using growing steers in one experiment the results showed a marked degree of discriminate grazing of the herbages. The forage selected by the grazing animals was higher in crude protein, ether-extract, and mineral matter, and lower in crude fiber than the whole clipped herbage available for consumption. All the constituents of grazed herbage were more digestible than those of the clipped herbage fed to confined steers. These workers concluded that the chemical composition of clipped herbage was an unreliable index of the chemical composition of herbage selected by grazing animals.

2. The Hand-Plucking Technique

Cook and Harris (25) collected forage samples by careful observation of grazing animals and hand-plucking forage comparable to the material being grazed. Forbes (40) discussed the complexity of obtaining a representative sample of forage actually grazed by an animal. With simple forage species at a uniform stage of maturity, he stated that hand-plucking was reasonably reliable. The error in sampling increased directly with the heterogeneity of the herbage available.

3. Use of the Esophageal-Fistula in Sampling Forages

Lesperance and coworkers (57) used esophageal and

rumen-fistulated steers to sample grazed forage. They described the surgical operation on a steer to install a plastic esophageal fistula. Comparisons between feeds of known composition and samples collected from these same feeds through fistulas indicated that some changes in composition occurred. These changes in composition were influenced by the type of feed and type of fistula. The amount of protein and ether-extract was the same in the fistula samples as the feed, but significant changes were noted in the amount of crude fiber, nitrogen-free-extract and energy. Fistula samples were highly contaminated with mineral matter. The fistula sample could be separated botanically between grasses and broad-leaved plants.

Lesperance and coworkers (58) used both esophageal and rumen-fistulated steers to study selective grazing. Botanical analysis indicated, in all cases, that grass increased in the grazing animal's diet as the period progressed and, conversely, clover decreased. Protein decreased and crude fiber increased in fistula samples, with respect to time, even though the chemical composition of clipped samples remained fairly constant. The composition of fistula samples failed to agree with the composition of samples hand-harvested from under cages the same day.

Weir and Torell (81) studied selective grazing by sheep. Pooling all data for ungrazed forage indicated that esophageal-fistulated sheep, using the method of Cook and Harris (25), selected

forage containing 4.1 per cent more protein and 3.5 per cent less crude fiber than that found in hand-clipped forage. When the forage had previously been grazed by a large flock of sheep the differences were somewhat smaller. The difference for crude fiber between grazed and ungrazed forage were highly significant. Correlation and regression studies indicated that it was not feasible to estimate what a sheep would eat from hand-clipped material. Edlefsen et al. (35) found statistically significant differences between hand-plucked and esophageal-fistula samples from sheep for all constituents except ether-extract, total protein, and cellulose. The greatest differences were found in ash and phosphorous.

The esophageal-fistula technique of sampling pastures appears to be the method of choice, since the forage sample collected by this method represents what the animal actually grazes and not what is available on the pastures as measured by hand-clipped samples as well as hand-plucked samples (simulated grazing).

C. The Development and Use of the Artificial Rumen Technique in Forage Evaluation Studies

Forage digestion studies using cattle or sheep have often been used by research workers for the nutritional evaluation of pasture forages. This method measures the availability of nutrients in plants for animals by the determination of the chemical composition of the plants and the digestion coefficients of the nutrients present. The

conventional method of digestion studies (using feeding trial and indicator methods) is not usually convenient to run and is laborious, time-consuming, and expensive because of the need and care for several animals (13, 25, 69). Digestion coefficients determined by feeding trials are influenced by several factors (60) and could vary between animals and under different feeding and management conditions. Thus, a more convenient and accurate method of determining the digestibility of pastures for dairy cattle remains to be developed.

1. Development of the Artificial Rumen Technique

Studies of the role of rumen microorganisms in the nutrition of ruminant animals have led to the development of the "artificial rumen" or in vitro rumen fermentation technique. Marston (59) was probably the first to study the metabolism of rumen microorganisms by culturing the population of rumen microorganisms in vitro and determining the end-products formed. Burroughs et al. (15) developed a laboratory technique or artificial rumen similar to the one used by Marston for purposes of studying the physiology of rumen microorganisms and the nutritional factors involved in the microbial digestion of roughages in the rumen. Using the above technique, Burroughs et al. (16) reported that the microbial digestion of poor quality roughages was greatly improved by the addition of available nitrogen, a complex mineral mixture, and an autoclaved water extract of cow manure. The nutritional requirements of the rumen bacterial population have been studied

by the same workers (5, 17, 18) with the result that rumen microorganisms have been shown to require a) an anaerobic atmosphere, b) minerals, c) a source of ammonia nitrogen, d) cellulose, and 4) soluble carbohydrates. Bentley et al. (11) studying the cellulolytic factor activity of rumen juice for rumen microorganisms in vitro, reported that valeric and caproic acids and to a lesser extent isobutyric and iso-valeric acids markedly increased the rate of cellulose digestion and ammonia utilization. Biotin and para-amino benzoic acid were also required by the microflora for maximum cellulose digestion. Dehority et al. (30) isolated and identified compounds from autolyzed yeast, alfalfa meal, and casein hydrolysate and reported that the amino acids, valine, proline, leucine, and isoleucine were the compounds that were cellulolytically-active when used in in vitro rumen fermentations. With the use of artificial rumen technique it was possible to study the nutritional requirements for rumen microorganisms for their functional role in the digestion of feeds for ruminants.

2. Reliability of the Artificial Rumen as a Method of Determining the Nutritive Value of Forages.

The artificial rumen method by Pigden and Bell (66) was probably the first in vitro method used for evaluating forage quality. They used an impermeable system with buffer-mineral solution, calcium carbonate, one gram forage substrate and fresh rumen inoculum. Their data indicated a close agreement between carbohydrate digested

in vitro from 11 forages and the corresponding TDN values determined from conventional trials with sheep. Barnett (8) described an *in vitro* method of determining the digestibility of silage cellulose. He also used an impermeable system with mineral-salt solution and sheep rumen liquor for the inoculum. The results obtained on 27 different types of silage showed significant correlations with the corresponding crude fiber digestibilities obtained by feeding trial. Cason and Markby (21) studied the efficacy of several *in vitro* methods for estimating the nutritive value of forages. Among the laboratory methods used cellulose digestibility appeared to be the most closely related to the *in vivo* digestibility data of 11 forages.

Quicke *et al.* (67) described an artificial rumen technique which they used to measure cellulose digestion of forages. Little difference was noted when cellulose digestion was measured using strained juice, phosphate buffer extract, or resuspended ruminal microorganisms as inoculum. The authors observed no significant difference in cellulose digestibility obtained *in vitro* (48-hour incubation) and *in vivo* with seven grass hays. However, there was a significant difference in some of the legume hays studied. Using a similar *in vitro* method, Hershberger *et al.* (47) determined cellulose digestibility of 35 forages. Comparing this with digestibility of forages determined by sheep digestion trials, highly significant correlations ($r = .97$ and $.92$) were obtained with *in vivo* cellulose digestion and digestible energy of the same forages, respectively.

Reid et al. (70) reported highly significant correlations between in vivo and in vitro digestibility of dry matter, cellulose, fiber, energy, and protein of pasture grasses. In vivo digestibility was most accurately predicted from in vitro dry matter digestibility of oven-dried samples. Baumgardt et al. (9) compared the accuracy of several methods in evaluating forages and stated that the estimates of TDN by the method of Pigden and Bell (66) were significantly correlated but consistently lower than the animal digestibility data. Forage cellulose digestion in the artificial rumen was significantly correlated with TDN (in vivo method) as well as with the digestion coefficients for dry matter, organic matter, and energy. Baumgardt et al. (10) modified the artificial rumen of Pigden and Bell (66) and used an impermeable system containing McDougall's artificial sheep saliva. The per cent forage cellulose digested with this method was significantly correlated with TDN, dry matter, and digestible energy, as well as the digestion coefficients of energy determined in vivo. The inclusion of a standard forage in each in vitro digestion trial allowed adjustments to be made so that cellulose digestion obtained in two different trials had a coefficient of variation of only 1.59 per cent. Donefer et al. (32), using the artificial rumen procedure described by Quicke et al.(67), studied the rate of cellulose digestion of nine forages. When compared to the leguminous species, grasses displayed lag periods in the start of cellulose digestion. These lag periods were reflected in the 12-hour

cellulose digestion determination, the latter being highly correlated ($r = 0.91$) with the Nutritive Value Index proposed by Crampton et al. (28). This index takes into consideration both the intake level and availability (digestibility) of energy of a forage and should therefore be a more accurate method of measuring the effective feeding value of forages. Thus it was proposed that the Nutritive Value Index (Y) of a forage may be predicted from the 12-hour in vitro cellulose digestion (X) of that forage according to the equation, $Y = -7.8 + 1.314X$.

Therefore, there is sufficient evidence that the artificial rumen technique offers great promise in predicting the nutritive value of pastures and forages for ruminants. The determination of cellulose digestion has been found to be an accurate measure of the availability of energy of forages and can be used in the prediction of the Nutritive Value Index proposed by Crampton (28)..

3. Factors Affecting Artificial Rumen Experiments

According to Burroughs et al. (16) there are limitations in the experimental use of the artificial rumen compared with studies in ruminant animals. The most important of these limitations is whether the conditions set up in the laboratory are truly representative of the conditions which occur under natural conditions in the live animal. Approximations must be made in the artificial rumen such as the addition of chemical constituents which appear similar to saliva, the

method of controlling end products of fermentation such as organic acids which are constantly being absorbed in the rumen, and the types of microorganisms which develop in the artificial rumen as compared to the microflora maintained in the rumen proper. These are only a few among many other factors that are involved in artificial rumen experiments.

Warner (79) expressed doubt on the validity of in vitro studies with rumen microorganisms in artificial rumen systems when the test substrate used in vitro was different from the diet fed to the animal from which the rumen inoculum was taken. He studied several criteria of normal rumen function in experiments with the artificial rumen using a semi-permeable system and reported that chemical criteria such as the rates of digestion of substrate or of production of metabolites can be quite sensitive over short periods of time (about 8 hours).

Johnson et al. (51), using an improved inoculum for in vitro rumen fermentations by discarding the first extraction of the rumen contents and resuspending the pressed pulp in a buffer solution, obtained greater cellulose digestion and less variation between experiments. They also reported that a mixture of acetic, propionic, and butyric acids (300, 300, and 30 mg. per 100 ml., respectively) inhibited cellulose digestion, but that those were above levels usually produced in 30-hour in vitro rumen fermentations.

In a comparison of cellulose digestion coefficients in vitro and

in vivo with 22 different rations, LeFevre and Kamstra (56) reported that the 48-hour in vitro fermentation period most nearly simulated the in vivo cellulose digestion. Per cent cellulose digestion values in vitro using sheep and cattle rumen fluid inocula were similar and highly correlated ($P < .01$). However, when the animals which were used to obtain rumen inoculum were on different rations, the cellulose digestion values in vitro were of different magnitudes. During the spring of the year Clark and Mott (24) obtained in vitro digestibility estimates of forages that were significantly correlated ($r = .72$) with data obtained on the same forages in conventional digestion trials. However, when the in vitro digestion trials were repeated in the fall, the estimates were low and no longer significantly correlated with the animal data. They suggested that the low values in the fall were due to differential effects of the sources of inoculum. Asplund et al. (6) and Hunt et al. (50) reported qualitative and quantitative differences in vitro for rumen liquors from steers fed different diets. These findings would indicate that marked changes from animal diets to in vitro test substrates would result in an extreme shift in microbial population in view of the differences in cellulolytic microorganisms in rumen samples as noted by Bryant and Burkey (14). On the other hand, Stewart and Schultz (75), Salisbury et al. (72), and Quicke et al. (67) observed little difference in cellulose digestion by various rumen liquor sources even though different techniques were employed. It would seem that the

preparation of the rumen inoculum and the removal of substances from the inoculum might also be an important factor affecting digestibility determinations in the artificial rumen when donor animals are fed different diets.

Church and Peterson (23) tested the effects of several variables commonly encountered during in vitro rumen fermentations. They suggested that if data from artificial rumen experiments are "to be extrapolated to other conditions and other laboratories," a standard procedure should be developed taking into account the different variables encountered. They indicated that rumen liquor sources, pH adjustments, quantity of rumen liquor and substrate, and particle size should be standardized within and between laboratories. Certainly there are other factors such as length of fermentation, addition of minerals, and the effect of various techniques that should also be considered.

El-Shazly et al. (37) studied the effects of an all-glass (impermeable system), semi-permeable membrane, and continuous flow types of artificial rumen on cellulose digestion, volatile fatty acid and ammonia production from purified cellulose and forages. No major differences were found between the different types of apparatus although they indicated that the all-glass system appeared to be advantageous because of its simplicity. This all-glass type of artificial rumen has been used in many studies on the nutritional requirements of rumen microorganisms for cellulose digestion (11, 30,

51) and also in studies on the reliability of the artificial rumen technique of forage evaluation (47, 67). Using this same type of apparatus, El-Shazly et al. (37) made biochemical and microscopic comparisons of in vivo and in vitro rumen fermentations since some workers (15, 79) have doubted the authenticity of cellulolytic microorganisms in vitro especially when fermentations were carried out for long periods. The rates of cellulose digestion and production of volatile fatty acid end-products were similar in vivo (nylon bag technique) and in vitro. No marked morphological change in bacterial population were observed from in vivo and in vitro rumen fermentations which were carried out for periods of 24-30 hours.

In view of all the work done to test the reliability of the artificial rumen technique in the measurements of nutritive value of different forages, it is safe to assume that in vitro rumen fermentations would be representative of those occurring in vivo so far as digestibility determinations with forages are concerned. The in vitro procedure should be standardized, however, in reference to those factors that were discussed in this review. Clark and Mott (24) suggested that "all lines to be screened should be included in a single trial to ensure maximum control of variables associated with the technique."

4. Significance of the Volatile Fatty Acids Produced in Vitro in Forage Studies

It is now established that the principal sources of energy for ruminants are the volatile fatty acids (acetic, proponic, butyric, and higher acids) and lactic acid which are the end-products of microbial digestion of dietary carbohydrates (mainly from roughage) in the rumen and which are readily absorbed through the rumen wall. Phillipson and Cuthbertson (65) have shown that at least 600-1,200 Calories of energy are absorbed as VFAs from the sheep rumen every 24 hours. Similarly in cattle, Carroll and Hungate (20) have shown that 6,000-12,000 Calories become available from the VFAs produced by fermentation in the rumen. The total energy turnover of fasting adult sheep and cattle is about 1,100 and 6,500 Calories respectively per day, indicating that VFAs make a major contribution to the energy requirements since it is recognized that these acids are utilized by body tissues (4).

This recognition of the importance of VFAs as major sources of energy to the ruminant has resulted in much interest on the problem of measuring the amounts and rates of production of VFAs in the rumen of cattle and sheep under various feeding regimes (7, 38, 72, 75, 80). However, Annison and Lewis (4) expressed doubt on the validity of VFA data based on ruminal VFA concentrations. They stated that the concentration of a particular acid or any rumen metabolite at any one time

is dependent on the rates of (a) production in the rumen, (b) absorption from the rumen, (c) passage from the rumen to the omasum, (d) dilution with saliva, (e) utilization by rumen microorganisms, and (f) conversion to other rumen metabolites. In addition it has been established that the individual VFAs are absorbed from the rumen at different rates which are partly dependent on the concentration of the acid, and on the pH of the rumen (41, 63).

The artificial rumen offers an alternative approach to the problem of measuring VFA production by rumen microorganisms from various feeds. Gray et al. (42) quantitatively measured the in vitro production of VFAs and methane by rumen microorganisms from two kinds of hay. The percentages of acetic, propionic, and butyric acids produced from "wheathen" hay were 41, 31, and 16 per cent, respectively, whereas those of "lucerne" hay were 53, 29, and 18 per cent, respectively. Evidence indicated that the amount and nature of VFA production in vitro may be related to forage quality. Barnett and Reid (8) studied VFA production of fresh grass in the artificial rumen at different stages of the grazing season. The main acid produced in early stages of the year was acetic acid, but as the season advanced propionic acid was produced in greater amounts. Using dried grass samples corresponding to the fresh material, acetic acid was produced in greater proportion than propionic acid. It was suggested that these variations between the fresh and dried grass were due to

changes in carbohydrate content resulting from storage of the latter. However, the authors did not relate the VFA production trends during the grazing season with other measures of pasture quality or animal response.

Asplund et al. (6) determined dry matter loss and VFA production of forages in the artificial rumen and obtained a high degree of correlation between dry matter digestibility in vivo (sheep trial data) and both dry matter loss and VFA production in vitro of the same forages. These were also highly correlated with the crude protein contents of hays. Stewart and Schultz (75) studied the in vitro VFA production (12 hour incubation period) from various dairy feeds by bovine rumen microorganisms. They reported that urea consistently increased VFA production in vitro regardless of the substrates used, although in subsequent in vivo experiments it increased VFA production only slightly. Fresh hand-clipped legume mixed grass caused a greater VFA production than did the legume hay. The grass markedly depressed propionic acid formation compared to legume hay.

In a study of the effect of six different rations on the in vivo and in vitro production of VFAs by rumen microorganisms, Hinders and Ward (48) reported that the greatest in vitro production of VFA occurred when the substrate consisted of the same hay as that in the ration of the animal from which rumen inoculum was used. However, in vitro total VFA, pH, and the acetate/propionate ratio were

lower than those in rumen fluid (in vivo). These results support the contention of Annison and Lewis against the validity of VFA production data determined from actual rumen contents.

There are certain limitations and assumptions to be made with the artificial rumen as a method of determining the nutritive value of forages for ruminants. The more important of these include the assumption that a) cellulose digestion and VFA production in vitro proceed at a rate similar to those which occur in the rumen, b) that the normal microbial population and activity are maintained during the incubation period and c) that the accumulation of metabolic end-products (in permeable systems) do not inhibit the fermentation reactions (4). However, the use of the proper in vitro technique should allow for conditions to occur similar to those in the rumen. Sufficient evidence has been presented whereby various artificial rumen techniques have been successfully applied in the evaluation of forages and the results obtained have shown a high degree of relationship with those obtained from in vivo work. The in vitro technique also offers certain advantages over in vivo methods which have already been discussed.

III. MATERIALS AND EXPERIMENTAL METHODS

A. Materials

1. Pastures

This study was conducted at the Iberia Livestock Experiment Station, Jeanerette, Louisiana. Permanent pastures, adequate in size for summer grazing by the station herd, were used for this study.

A five-year pasture rotation program was being carried out at the station. Oats, ryegrass and S-1 clover together with Bermuda grass and Dallis grass were seeded in the first year, with reseeding clovers, volunteer grasses and sod-seeded oats and ryegrass on the second, third and fourth years. The pastures were plowed under on the fifth year and seeded to Alyce clover for hay. The soil was a silty clay loam.

2. Animals

The station herd has consisted of Jerseys, Holsteins, Sindhi-Jersey and Sindhi-Holstein crosses, and three-way crosses among these breeds. The cows were being fed a 16-18 per cent concentrate feed during each of two milkings at the rate of one pound per day for every three pounds of milk for the Jerseys and Sindhi-Jersey crosses,

and one pound for every four pounds of milk for the Holsteins and Sindhi-Holsteins. The herd was grazing the permanent pastures as the only source of forage.

Ten cows consisting of Holsteins, Jerseys, Sindhi-Holstein, Sindhi-Jersey and three-way crosses, were selected from the herd. They were within 40 to 120 days of their lactation period at the beginning of the experiment. Milk production data of these cows during the experimental period were used for the prediction of milk yield.

Two culled, dry cows (one Holstein and one Jersey) were operated on at Louisiana State University to install stainless steel esophageal-fistulas according to the method described by Rusoff and Foote (71). These cows were brought to the Iberia Station and were used to obtain esophageal-fistula pasture samples from the experimental pastures.

The experimental period started on June 1, 1960 and was carried on for 120 days up to September 28, 1960.

3. Climate and Other Environmental Conditions

The summer weather at the station was mostly hot and humid with maximum and minimum ambient air temperature averaging about 90 degrees F. and 70 degrees F., respectively. There was hardly any rainfall during the first part of the study period (June) but there was ample rainfall in July and August. Mosquitoes and flies were abundant

on the pastures. There was sufficient shade from trees for the grazing herd.

B. Experimental Methods

Forage samples to be used for chemical analyses and digestion experiments with the artificial rumen were obtained weekly from the pastures by hand-plucking and by means of the esophageal-fistulated cows. Preliminary digestion experiments were conducted with the artificial rumen to standardize the artificial rumen procedure. All determinations made from the forage samples including pasture quality score, climatic and milk production data were studied and statistically analyzed.

1. Collection of Hand-Plucked and Esophageal-Fistula Pasture Forage Samples

Forage samples were collected in the morning and in the afternoon once weekly while the station herd was grazing on the pastures. Forage samples were collected following grazing cows, and hand-plucking as representative a sample as what the cows were actually grazing. The cows were followed for about two hours during each collection.

Esophageal-fistula forage samples were obtained from two dry cows, one Holstein and the other a Jersey, which were also following the grazing herd during the time of sample collection. These animals

were fitted with stainless steel esophageal-fistulas according to the method of Rusoff and Foote (71). A canvas bag was harnessed on the cow underneath the fistula. It was necessary to fast the cows for a day or overnight before each collection. This permitted the cows to graze continuously during the time of collection and minimized the amount of regurgitated materials flowing out of the fistula.

The forage samples were placed in plastic bags, sealed, transported to Baton Rouge in dry ice and stored in a deep freeze refrigerator. A total of 17 weekly samples were collected by the hand-plucked technique and an equal number by the esophageal-fistula technique.

2. Preparation of Forage Samples

After all forage samples were collected during the 17-weekly study period and stored, the frozen samples were thawed out. The morning and evening samples were mixed. Duplicate one-kilogram portions of each weekly composited sample were oven-dried at 70 degrees C. and the dry matter content was determined. The samples were then ground through a No. 20 mesh in a Wiley mill. A composite of each ground sample was kept in two properly labeled half-pint glass jars.

Forage samples to be used for artificial rumen digestion were reground through a No. 40 mesh screen in a small table-type Wiley mill. They were then oven-dried at 65 degrees C. for 24 hours, and kept in a desiccator.

3. The Artificial Rumen Procedure

A modification of the method of Cheng et al. (22) was used for the artificial rumen digestion of the forage samples. Rumen contents obtained from fistulated steers were strained through eight layers of cheese cloth and collected into a two-quart-size thermos bottle fitted with stainless steel lining. Twelve hundred milliliters (ml.) of liquid was centrifuged in a Servall high-speed angle centrifuge at about 1,000 r.p.m. for one minute and 45 seconds. By this process partially digested feed particles in the liquid as well as most of the protozoa were sedimented and discarded. The supernatant was then centrifuged again at a speed of 5,000 r.p.m. for 20 minutes. The resulting sediment consisting principally of rumen bacteria was suspended in 400 ml. of 0.8% sodium bicarbonate buffer solution adjusted to pH 7, and centrifuged again for 20 minutes at 5,000 r.p.m. The final sediment was suspended in 400 ml. of bicarbonate solution.

Two-tenths of a gram of oven-dried sample was weighed in duplicate and transferred into 50 ml. Erlenmeyer flasks fitted with rubber stoppers and inlet and outlet glass tubings for passage of carbon dioxide gas. Fifteen ml. of one per cent bicarbonate buffer was pipetted into each sample flask and stirred with the substrate on a magnetic stirrer for three minutes. The substrate media were kept refrigerated overnight to allow the substrate to presoak in the buffer medium.

Prior to inoculation of the flasks the following morning, the substrate media were placed in a constant temperature water bath, adjusted to 39.5 degrees C. and bubbled with carbon dioxide so as to adjust the pH of media to about pH 7.1. No readjustment was required during the course of a 48-hour incubation period, because at the end of the incubation period the pH of the flasks did not go below pH 6.5. An aliquot of 10 ml. of the prepared cell suspension was pipetted into each sample flask making a total volume of 25 ml. Two flasks containing duplicate samples were inoculated at a time and were set on the water bath, the gas outlet tube of the first flask connected with a rubber tubing to the inlet tube of the second flask (Figure 1). Carbon dioxide was bubbled continuously at a slow rate during the 48-hour incubation period. The gas served to provide an anaerobic condition as well as to agitate the solution. At the end of 48 hours, the digestion flasks were taken down, the contents transferred quantitiatively into 75 ml. glass centrifuge tubes and centrifuged at 1000 r.p.m. for five minutes. A portion of the supernatant liquid was transferred into a glass vial fitted with screw cap, acidified with 50 per cent sulfuric acid to about pH 2, and frozen for subsequent volatile fatty acid analyses. The sediment in each tube was used to determine cellulose content by a modification of the method of Crampton and Maynard (29).

Briefly the method used to determine cellulose content is

described as follows: After decanting the supernatant liquid from the centrifuge tubes, the remaining sediment in the tubes was digested in a boiling water bath with 25 ml. of 1.25 per cent sodium hydroxide (NaOH) for exactly 20 minutes. Then the samples were centrifuged immediately at 1000 r.p.m. for five minutes and the NaOH digest decanted and discarded. The samples were digested in boiling water bath with 12 ml. glacial acetic acid plus 2.5 ml. of concentrated nitric acid for exactly 20 minutes. Immediately after acid digestion the samples were filtered with suction in Gooch crucibles fitted with asbestos pads and washed three times with cold alcohol (95% ethanol), then three times with hot benzene, and finally three times with hot alcohol. The samples were oven-dried at 100 degrees C. for 4 hours, placed in a desiccator, and allowed to cool. They were weighed, ignited for at least two hours in a muffle furnace set at 650 degrees C. Finally, the ignited samples were allowed to cool in a desiccator, and were reweighed. Cellulose contents were determined by difference between the first and second weighings.

Thirty-six fermentation flasks were utilized in one digestion trial which permitted all 17 collections for each of the hand-plucked and esophageal samples to be run in duplicate including an alfalfa hay standard. This standard forage was used to correct for differences in digestibility values between the same set of samples from one trial to the next.

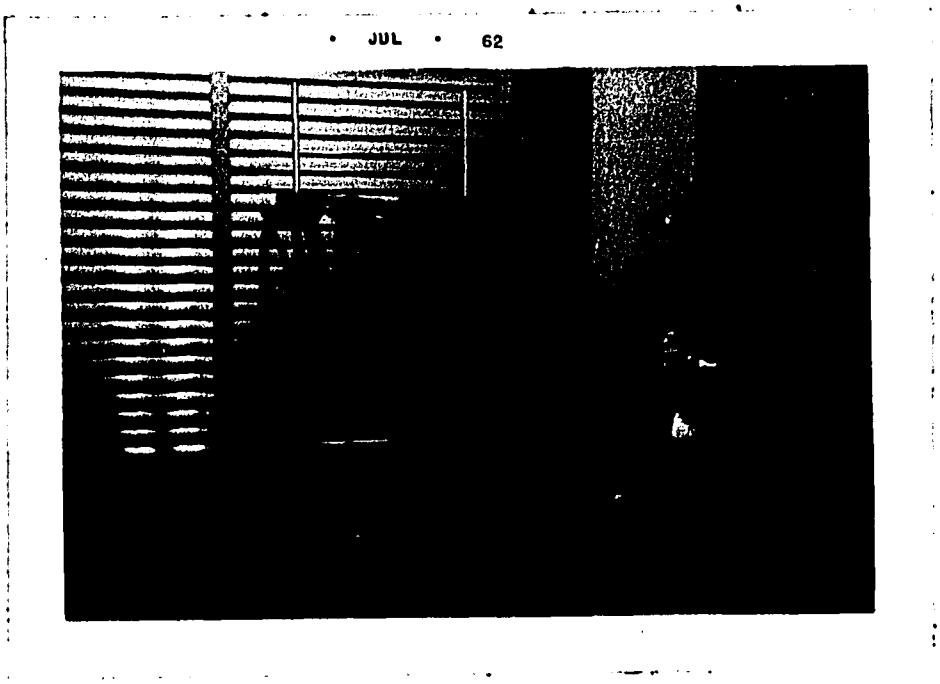

FIGURE 1. The Artificial Rumen Apparatus with 36 Digestion Flasks

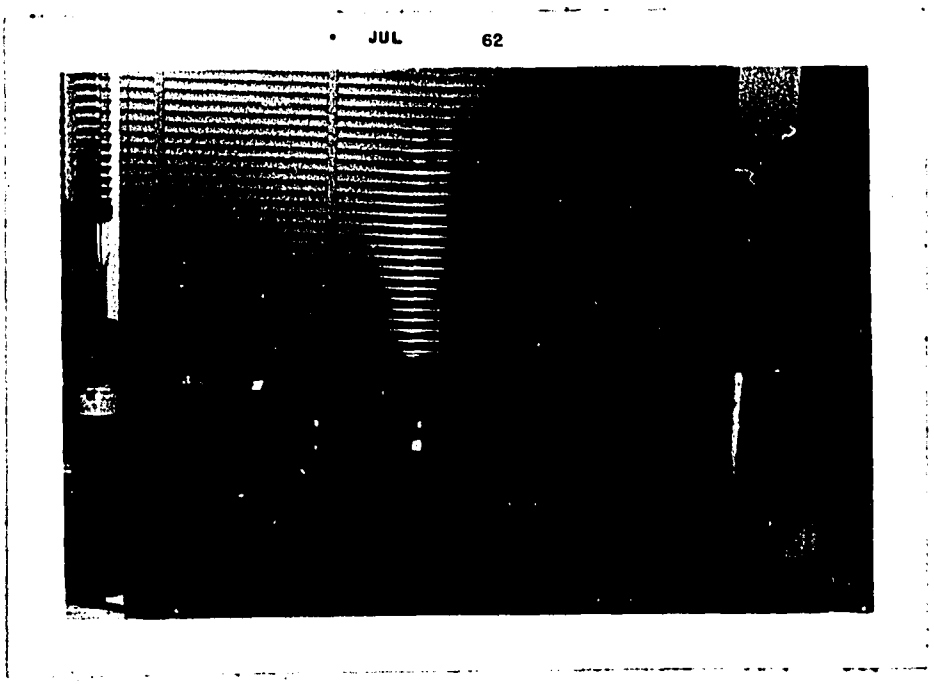

FIGURE 2. Equipment Used for Artificial Rumen Work

The artificial rumen setup that was made for this research is shown in Figures 1 and 2.

4. The Artificial Rumen Digestion Experiments

a. Preliminary In Vitro Experiments

Four preliminary digestion experiments were carried out to standardize the artificial rumen procedure, since no one procedure is suitable under various conditions, The use of two kinds of forage substrate--hand-plucked and esophageal-fistula forage samples--also required a standardized artificial rumen procedure to determine cellulose digestibility and volatile fatty acid production of the pasture forages.

The first experiment was designed to test the effect of weight of substrate and kind and amount of medium on forage cellulose digestibility. Since the concentration of substrate in the medium commonly used in artificial rumen studies is one per cent or less, three weights, namely, 0.15, 0.20, and 0.25 gram of forage substrate were used. Simultaneously, two kinds of medium at two concentrations (10 and 15 ml.) were also used with the three weights of forage substrate in a 24-hour digestion trial. This was a two by three factorial design, each treatment combination run in duplicate. The two media used were: sodium bicarbonate buffer (0.8%) and nutrient medium based on the method of Cheng et al. (22). The composition of the nutrient medium is given in Appendix Table 1a.

A second experiment was run to determine the effect of kind of medium and cell concentration on the in vitro cellulose digestibility of hand-plucked and esophageal-fistula forage samples. The two kinds of medium used in the first experiment were tested with 10 and 15 ml. of cell concentration in a 24-hour digestion trial.

The third experiment tested the effect of four initial pHs of the medium, namely, pH 6.75, 6.90, 7.10 and 7.40, on the in vitro cellulose digestibility of forages in a 24-hour digestion trial.

The fourth experiment was a time study to determine the rate of cellulose digestion of forages in a 48-hour in vitro digestion trial. Sample flasks were taken down at five eight-hour intervals and cellulose digestibility was determined at each time interval. Alfalfa hay and Solka Floc (purified cellulose) were included in the time study to compare their rates of digestion with the forage samples.

b. The In Vitro Forage Digestion Trials

Since it was necessary to find out whether or not the source of inoculum for the artificial rumen affected cellulose digestibility of forages, four 48-hour digestion trials were run using two sources of rumen inoculum, one from a rumen fistulated steer grazing on summer permanent pasture and the other from another fistulated steer being fed alfalfa hay and 3 pounds of concentrate on dry lot daily. Two of the four digestion trials were run on hand-plucked samples, and the other two trials on esophageal-fistula samples, and two different sources of rumen inoculum were used for each of the above two trials.

To determine the weekly trends in the cellulose digestibility of hand-plucked and esophageal-fistula pasture samples, four 48-hour digestion trials were conducted using two trials for each of the two methods of sample collection. All 17 weekly-collected forage samples by each method of collection were run in duplicate in each digestion trial.

5. Chemical Analyses of Forage Samples

The proximate analyses (crude protein, crude fat, crude fiber, ash, nitrogen-free extract and moisture) of the forage samples were determined by the procedures recommended by the Association of Official Agricultural Chemists (1). The analyses for calcium, phosphorus, sodium, and potassium of the forage samples were also made according to A.O.A.C. methods (1).

Cellulose analysis of the forage samples was carried out according to the procedure of Crampton and Maynard (29) with modifications as described previously.

Volatile fatty acid determinations were made on the liquid portion of the artificial rumen digested forage substrates by the chromatographic method of Keeney (55) with minor modifications. The modified method is described briefly as follows:

Two milliliters of the acidified liquid sample from the artificial rumen digestion was mixed homogeneously with three grams of dry silicic acid (Mallinckrodt's No. 2847) and transferred into a

previously prepared chromatographic column made of the dry silicic acid which had been mixed with an ethylene glycol solvent and then slurried with one per cent butanol-hexane. The slurry was packed under five pounds-per-square-inch applied air pressure in a glass column, 40 centimeters by 20 millimeters and fitted with a teflon stopcock and a drip tip on one end, and a wide glass reservoir fused on the other end.

Successive small portions of one per cent butanol in hexane were allowed to flow through the sample to extract and develop the fatty acids. After about 100 ml. of the mobile solvent had passed through the sample, butyric and propionic acids developed as distinct yellow bands. After 150 ml. of solvent had flowed through the sample, butyric acid was eluted from the column and propionic acid was near the elution point. This time, 150 ml. of five per cent butanol in hexane was allowed to elute the propionic acid and about 300 ml. eluted the acetic acid, which showed the largest yellow band. Each acid fraction was collected in separate flasks and immediately titrated with 0.01 N standard alcoholic potassium hydroxide to the thymol blue end-point. Eleven chromatographic columns were used in every run. One column was used for a blank to correct for carbon dioxide contamination from the air and the butyric acid impurities in the solvents used.

The fatty acids (acetic, propionic, and butyric) were calculated as micromoles of acid in two ml. of digested sample (from the artificial rumen) and then converted to milligrams per gram of forage sample.

The glycol solvent was prepared by dissolving 700 milligrams of brom cresol green in 700 ml. of ethylene glycol, and mixing this with 296 ml. of distilled water, heating over a water bath until the brom cresol green was completely in solution. After cooling, 4.0 ml. of N ammonium hydroxide was added. This prepared ink-blue solvent was mixed with silicic acid in a mortar at the rate of about 0.9 milliliters of solvent per gram of silicic acid.

6. Pasture Quality Score and Climatic Data

Pasture quality score as described by Bertrand (12) and used for farms in Dairy-Herd-Improvement Associations, was made daily on the experimental pastures at the station by the same individual throughout the entire experimental period. The instruction sheet for the pasture score system is presented in the Appendix.

Maximum and minimum ambient air temperatures (degrees F.) were recorded daily. The amount of rainfall in inches was also recorded daily at the station during the experimental period.

7. Statistical Analyses

The preliminary experiments with the artificial rumen were statistically analyzed by analysis of variance according to the methods of Snedecor (74).

Multiple regression and correlation analyses were carried out on all variables using the statistical programs for the IBM 1620 as outlined

by Harkins (45) at the Louisiana State University Computer Research Center. The dependent variable, Y, was determined from the milk yield data of each of the ten selected cows from the Iberia Experiment Station herd. The persistency figure, 0.9144, as introduced by Corley (26) was used to calculate the expected lactation curve of each cow. The difference between the actual and expected milk yield (weekly average of each cow) was used as the Y value. The independent variables, Xs, consisted of the variables for the hand-plucked forages (X1 to X14) and the esophageal-fistula forages (X15 to X28), total ambient temperature (X29), total rainfall (X30), and pasture quality score (X31). Total ambient temperature is defined as maximum plus minimum temperatures (degrees F.). The Xs for hand-plucked and esophageal-fistula, respectively, are as follows: X1 and X15, *in vitro* cellulose digestibility; X2 and X16, *in vitro* acetic acid production; X3 and X17, propionic acid production; X4 and X18, butyric acid production; X5 and X19, total volatile fatty acid production; X6 and X20, acetate: propionate ratio; X7 and X21, crude protein content; X8 and X22, crude fiber content; X9 and X23, crude fat content; X10 and X24, nitrogen-free-extract; X11 and X25, ash content; X12 and X26, calcium content; X13 and X27, phosphorus content; X14 and X28, potassium content.

IV. RESULTS AND DISCUSSION

A. Preliminary Experiments with the Artificial Rumen Procedure

1. Effect of Weight of Substrate and Kind of Medium

Using the artificial rumen procedure described in this study, it was necessary to determine the weight of forage substrate and the proper medium to use for optimum digestion of forage cellulose. The results are presented in Table 1, and the analysis of variance for this experiment in Appendix Table 2a. It can be noted in Table 1 that 0.2 gram weight of forage substrate gave higher percentages of cellulose digestibility (except treatment II) than those of 0.15 and 0.25 gram weights of forage substrate. It can also be noted that treatments I and IV, both using a buffer medium, gave much higher percentages of cellulose digestibility of the forage than treatments II and III, using nutrient medium based on the method of Cheng et al. (22). There appeared to be no advantage in increasing the amount of medium from 10 to 15 milliliters. The analysis of variance of this experiment (Appendix Table 2a) showed that there were highly significant ($P < .01$) differences in per cent cellulose digestibility using the two kinds of medium and the three different weights of forage substrate. A highly significant ($P < .01$) interaction also existed between kind of medium and weight of forage substrate.

TABLE 1

Effect of Weight of Substrate and Kind of Medium on *In Vitro* Cellulose Digestibility of Forage[a]

	Treatment	Weight of Substrate in Grams .15	.20	.25
		% Cellulose Digestibility		
I	10 ml. buffer 10 ml. cell inoculum	27.6[b]	28.6	24.0
II	10 ml. nutrient medium 10 ml. cell inoculum	12.4	12.3	10.4
III	15 ml. nutrient medium 10 ml. cell inoculum	9.7	12.4	6.9
IV	15 ml. buffer 10 ml. cell inoculum	12.6	30.7	11.8

[a] Twenty-four hour incubation period
[b] Average of duplicate samples

TABLE 2

Effect of Kind of Medium and Cell Concentration on *In Vitro* Cellulose Digestibility of Hand-Plucked and Esophageal-Collected Forages[a]

	Treatment	Forage Hand-Plucked	Esophageal using Holstein cow	Esophageal using Jersey cow
		% Cellulose Digestibility		
I	10 ml. buffer 10 ml. cell inoculum	54.5[b]	2.8	51.1
II	10 ml. nutrient medium 10 ml. cell inoculum	47.7	7.1	49.2
III	10 ml. nutrient medium 15 ml. cell inoculum	53.7	10.8	55.7
IV	10 ml. buffer 15 ml. cell inoculum	58.6	9.0	55.4

[a] Twenty-four hour incubation period
[b] Average of duplicate samples

The 0.8 per cent buffer medium proved to be satisfactory for microbial digestion of forage cellulose. The medium had only to be adjusted to pH 7.1 by bubbling with carbon dioxide prior to inoculation of the artificial rumen. In this medium the rumen microorganisms depended entirely on the forage substrates under study for their food. Thus it was possible to determine the potentiality of the weekly-collected pasture samples to provide the necessary nutrients for optimum microbial activity in the artificial rumen.

Cheng et al. (22) reported that the concentration of cellulose used in the medium affected the amount of its digestion in 24 hours. Favorable digestion was obtained when concentration of cellulose in the medium was less than one per cent. The concentration of cellulose in the forage that showed optimum digestibility in the preliminary experiment of this study was about one per cent.

2. Effect of Kind of Medium and Cell Concentration

The effects of the two kinds of medium (buffer and nutrient) and two cell concentrations (10 and 15 ml.) on the cellulose digestibilities of one hand-plucked and two esophageal-fistula forage samples were determined in the artificial rumen. The results are presented in Table 2 and the analysis of variance in Appendix Table 3a.

The mean percentages of cellulose digestibility as seen in Table 2, were higher under Treatment I (buffer medium) for the hand-plucked forage (54.5%) and Jersey esophageal-fistula forage (51.1%) than those

of Treatment II (nutrient medium). The mean per cent cellulose digestibility was also higher under Treatment IV (buffer medium) for hand-plucked forage (58.6%) than that of Treatment III (nutrient medium). The mean percentages of cellulose digested under Treatments III and IV (15 ml. cell inoculum) were correspondingly higher than those under Treatments I and II (10 ml. cell inoculum) for all three forages. It can be seen from the analysis of variance (Appendix Table 3a) that significant differences ($P < .05$) existed between the effects of two kinds of medium and two cell concentrations (Treatments I, II, III, and IV) on the per cent cellulose digestibility of the forages. In addition, highly significant differences ($P < .01$) occurred in the percentages of cellulose digestibility of the three different forages. There was no significant interaction between treatment medium and kind of forage.

The esophageal-fistula forage from the Holstein cow gave very low cellulose digestibility values as compared to those of the esophageal-fistula forage from the Jersey cow and the hand-plucked forage. It was decided that the esophageal-fistula forage from the Holstein cow was probably greatly contaminated with salivary and rumen fluids. The forage that this animal grazed did not readily flow out of the fistula so that large amounts of fluid materials were collected. These materials could have caused unfavorable conditions for microbial activity to occur in the artificial rumen. It was decided

therefore, not to use the esophageal-fistula forages from the Holstein in the in vitro forage digestion trials for the determination of the nutritive value of pastures in this study.

3. Effect of pH of Media

The effect of four pHs (pH 6.75, 6.90, 7.10 and 7.40) of the medium were studied on one hand-plucked and three different esophageal-fistula forages from the Jersey cow. The purpose of using three esophageal-fistula samples was to test whether the initial pH of the medium would have varying effects on the cellulose digestion of these forages that might contain varying concentrations of salivary materials. The results of the in vitro digestion trial are given in Table 3, and the analysis of variance in Appendix Table 4a. It can be seen in Table 3 that pHs 7.1 and 6.9 of the medium gave higher percentages of cellulose digestibility for all four forages than those of pHs 6.75 and 7.40. The analysis of variance shows only treatments (different pHs of medium) to give significant effects ($P < .05$) on cellulose digestibilities of forages.

In other in vitro rumen studies, pH adjustments have been in the range of 6.4 to 6.9. Wegner et al. (80) suggested a pH range of 6.4 to 6.9 for optimum cellulose digestion by rumen microorganisms. Church and Petersen (23) reported pH 6.5 to 6.8 to result in maximum cellulose digestion under the conditions of their experiment. However, they suggested that the optimum pH for cellulose digestion by rumen

TABLE 3

Effect of pH of Medium on Cellulose Digestibility of Hand-Plucked and Esophageal-Fistula Forages[a]

pH of Medium (Initial)	Jersey			
	Hand-Plucked	Esophageal (Jersey)		
		1[b]	2	3
	% Cellulose Digestibility			
6.75	39.0[c]	39.0	36.3	28.4
6.90	46.1	40.2	40.3	41.2
7.10	45.6	48.8	40.8	45.2
7.40	41.2	39.9	39.0	40.5

[a] Twenty-four hour incubation period
[b] One of three different esophageal-collected forages
[c] Average of duplicate samples.

microorganisms would appear to vary according to substrate and/or source of rumen liquor.

4. Rate of Digestion Experiment

Two hand-plucked forage samples collected on different weeks were used in this experiment to determine their rate of *in vitro* cellulose digestion and the length of incubation period that would allow maximum cellulose digestion of the forages. Alfalfa hay was included in the time study since it was used as a standard forage in the *in vitro* forage digestion trials. The difference in cellulose digestibility of alfalfa hay was used to correct for variabilities in cellulose digestibility of forage samples under study between digestion trials. Solka Floc (purified cellulose) was also included in the time study to compare the rate of digestion of a pure cellulose *in vitro* with cellulose as a fibrous constituent of forages. The rate of digestion curves are presented in Figure 3. Forage A had a faster rate of *in vitro* cellulose digestion than forage B at all time intervals. This would indicate that a forage that has a greater cellulose digestibility value than another forage will also result in a faster rate of cellulose digestion. Compared to the alfalfa hay standard, the two forages showed uniform rates of digestion up to 24 hours of incubation after which the rates of digestion decreased up to 40 hours. Maximum cellulose digestion of the forages including the alfalfa hay was reached at 40 hours with a very slight increase at 48 hours. Compared to Solka

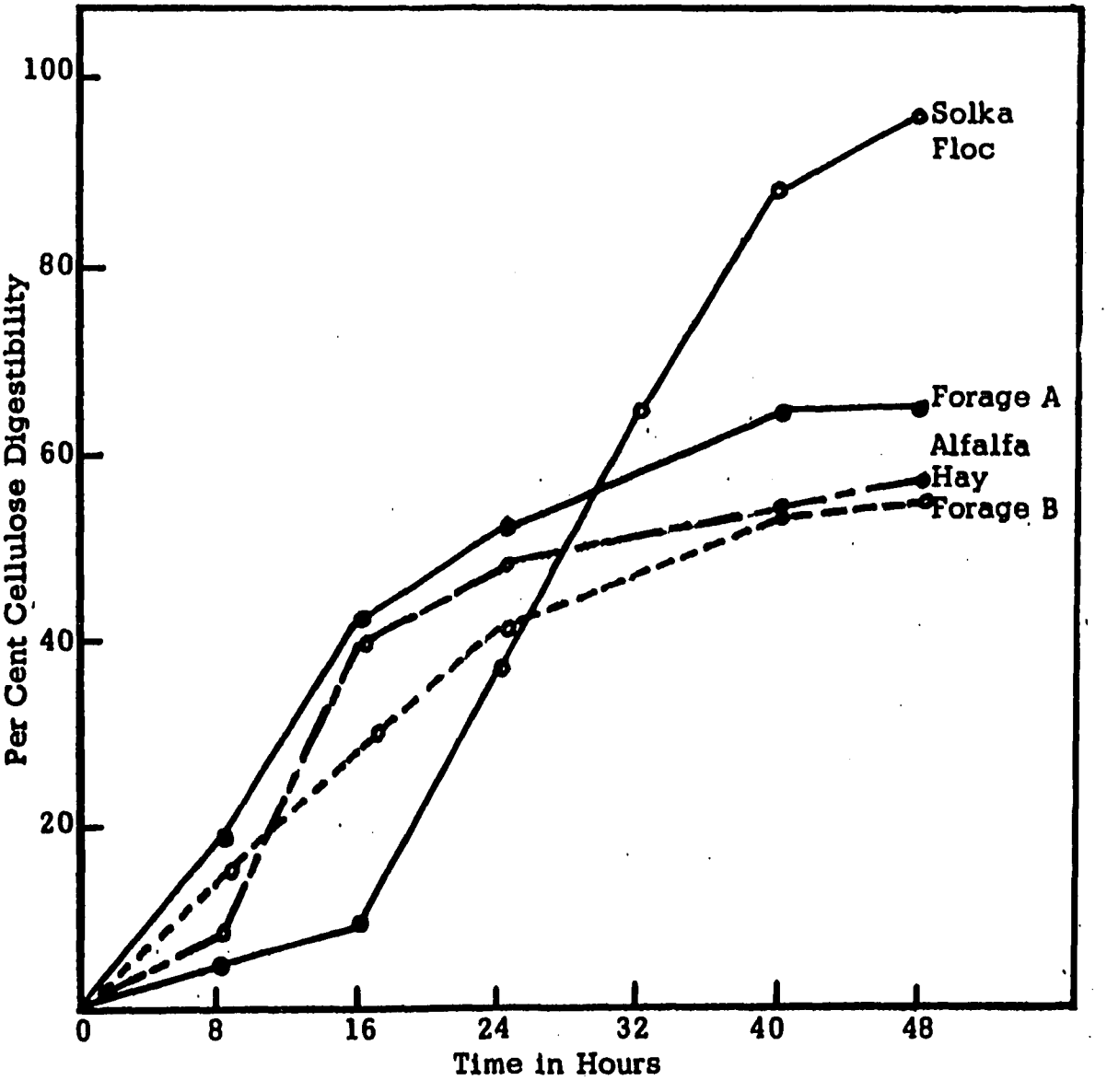

FIGURE 3. <u>In Vitro</u> Rate of Cellulose Digestion of Forages

Floc, the forages showed no lag phase while alfalfa hay had a lag at eight hours. However, after 16 hours of incubation Solka Floc exhibited a rapid rate of digestion up to 48 hours when it was almost 100 per cent digested. Kamstra et al. (54) studied the effect of stage of maturity and lignification on the in vitro cellulose digestion of forages. They reported that stage of maturity and the amount of lignin had a marked effect on the in vitro cellulose digestibility of the forages studied. However, when cellulose isolated from the same forages was used as the sole substrate in fermentation flasks, the digestibility was improved over the corresponding sample of the whole plant materials. They suggested that "encrusting substances could be involved (in the forages) since the isolated cellulose would be free of lignin or other encrusting substances indigenous to the whole plant materials." Salisbury et al. (72) reported that the holocellulose and "α-cellulose" portions of roughages showed more rapid and complete digestion than did the original plant materials.

5. Comparison of Two Sources of Rumen Inoculum

Two sources of rumen inoculum were used to determine the weekly trends in cellulose digestibility of the esophageal-fistula and hand-plucked pasture samples collected during the 17-week experimental period. One source of rumen inoculum was from a rumen-fistulated steer grazing solely on summer pasture and the other source was from another rumen-fistulated steer being fed alfalfa hay and three pounds

of concentrate daily on drylot. Two in vitro digestion trials were conducted on each of the hand-plucked and esophageal samples. The results are presented in Appendix Table 5a. Appendix Table 6a shows a highly significant correlation ($r = 0.887$, $P < 0.01$) in the weekly cellulose digestibility trends obtained for esophageal-fistula pasture samples using the two different sources of rumen inoculum. However, in the case of the hand-plucked pasture samples the digestibility trends obtained using the two different sources of rumen inoculum were not significantly correlated ($r = 0.331$) as indicated in Appendix Table 7a. It can be noted however, that only 12 weekly samples were used for the hand-plucked forages, whereas 17 weekly samples were used for the esophageal-fistula forages to compare the effects of two different sources of rumen inoculum.

B. Pasture and Climatic Data

1. Botanical Composition of the Pastures

In the beginning of the experimental period (June 1, 1960) the grasses on the pastures were mainly oats (Avena sativa) and ryegrass (Lolium multiflorum) in the mature stage. There was plenty of S-1 clover (Trifolium repens) and some other clovers such as California bur-clover (Medicago hispida) and spotted bur-clover (Medicago arabica). Bermuda grass (Cynodon dactylon) and Dallis grass (Paspalum dilatatum) were starting to grow at this time.

In the latter part of June and early July the predominant grasses were Bermuda grass and Dallis grass with some patches of clovers still remaining on the pastures. The pastures were dry during these weeks. In late July, and all through August, the conditions of the pastures improved probably due to the ample rainfall. The pastures had lush growth of Bermuda grass and Dallis grass and some Crabgrass (Digitaria sanguinalis). Towards the end of the experimental period (September 28, 1960) the principal grasses were Bermuda grass, Dallis grass, and Crabgrass, but the quality of the pastures was declining.

2. Chemical Composition of the Pastures

The average monthly chemical composition of the pastures from June 1, 1960 to September 28, 1960 is given in Table 4. Hand-plucked samples and esophageal-fistula samples collected from two cows were used in the chemical analyses.

The dry matter of the hand-plucked pasture samples was highest in June when the pastures consisted mainly of mature oats and ryegrass, and was lowest in July when Bermuda grass and Dallis grass were in the young succulent stage. Dry matter gradually increased in August and September as the grasses became more mature. The much lower dry matter content of the esophageal samples was due to the addition of saliva to the sample as the animals grazed and swallowed the forage material.

TABLE 4

Average Monthly Chemical Composition of the Pastures
(June 1, 1960 to September 28, 1960)

		June	July	August	September
		----------%			
Dry Matter	HP[a]	33.8	26.2	28.4	30.0
	EF[b]	12.6	11.6	13.3	15.4
Crude Protein	HP	12.4	12.9	14.3	11.4
	EF	12.7	13.8	13.6	11.1
Crude Fiber	HP	23.6	22.9	23.4	25.7
	EF	24.0	26.0	25.8	25.6
Crude Fat	HP	2.67	2.80	3.26	2.58
	EF	2.54	2.36	2.72	2.47
Nitrogen-free-extract	HP	44.3	44.4	42.9	47.4
	EF	40.5	39.9	41.0	41.1
Ash	HP	8.5	9.2	10.1	8.0
	EF	11.2	10.7	10.0	13.0
Calcium	HP	0.66	0.63	0.60	0.58
	EF	0.73	0.60	0.70	0.61
Phosphorous	HP	0.29	0.30	0.41	0.35
	EF	0.37	0.38	0.37	0.44
Sodium	HP	0.09	0.06	0.06	0.04
	EF	1.42	1.18	1.04	1.09
Potassium	HP	1.31	1.38	2.32	1.77
	EF	1.38	1.68	1.68	1.39

[a] HP refers to hand-plucked forages and the values for each chemical constituent are averages of weekly determinations.

[b] EF refers to esophageal-fistula samples collected from two cows and the values are averages of weekly determinations.

The hand-plucked samples showed increasing levels of crude protein from June, July and August and a decrease in September. Crude fiber decreased from June to July and increased in August and September. The esophageal-fistula samples did not show similar trends, probably due to the effect of selective grazing practiced by the animals. The crude fat content appeared to show trends similar to crude protein. The lower crude fat content of the esophageal samples might be due to the lipolytic action of saliva on the forage fat. There was probably some loss of soluble carbohydrates in the ingested forages which was indicated by the lower nitrogen-free extract content of esophageal-fistula samples.

The increase in ash content of the hand-plucked pasture samples from June to July and August was mainly due to the increases of phosphorous and potassium during the same months. The mineral contents of the esophageal-fistula samples did not follow the same trends because of mineral contamination from saliva. This was quite obvious for sodium analysis, where the esophageal samples were much higher in sodium than the hand-plucked samples. In general, the mineral contents of the pastures showed trends similar to those of crude protein.

3. Pasture Quality Score and Climatic Data

Table 5 shows the average monthly pasture quality score and the monthly climatic data. The average quality score in June was relatively high as compared with those of July and August when the pastures

TABLE 5

Average Monthly Pasture Quality Score, Ambient Air Temperatures and Total Rainfall during the Experimental Period

	June	July	August	September
Pasture Quality Score	20.2	14.9	19.4	18.0
Maximum Temperature, °F.	91.2	93.7	89.7	90.1
Minimum Temperature, °F.	69.0	73.8	72.9	68.4
Total Rainfall, Inches	3.91	5.14	14.82	2.95

TABLE 6

Average In Vitro Cellulose Digestibility, Acetic Acid, and Total Volatile Fatty Acid Production of the Pastures

In Vitro Determination[a]		June	July	August	September
Cellulose Digestibility, (%)	HP[b]	63.2	61.1	68.3	59.5
	EF[c]	63.6	62.0	62.4	55.8
Acetic Acid Produced, (mg. per gram of forage)	HP	165.5	180.6	190.5	180.9
	EF	115.8	118.0	138.3	133.3
Total Acid Produced (mg. per gram of forage)	HP	327.8	345.0	349.9	325.7
	EF	222.9	231.5	248.5	237.5

[a] In vitro determinations were obtained from two 48-hour digestion trials.

[b] HP refers to weekly-collected hand-plucked forages.

[c] EF refers to weekly-collected esophageal-fistula samples.

were in better condition. This was indicated by the chemical composition of the pastures as presented in Table 4. The average monthly ambient air temperatures did not change much except in July when there were days when the ambient temperatures reached 95 degrees F. or higher. There was much rainfall in July and August when the pastures showed much green and succulent growth.

4. Data Obtained from the In Vitro Forage Digestion Trials

a. Pasture Cellulose Digestibility

The weekly in vitro cellulose digestibility of the pastures was determined from hand-plucked and esophageal-fistula forages in four 48-hour digestion trials. Rumen inoculum for these trials was obtained from two rumen-fistulated steers that were being fed alfalfa hay plus three pounds of concentrate each on drylot. The following formula was used to calculate cellulose digestibility:

$$\text{Cellulose digestibility (\%)} = 100 - \left[\frac{\text{gram undigested cellulose in forage}}{\text{gram cellulose in forage}} \times 100\right]$$

Table 6 shows the weekly in vitro cellulose digestibility of the pastures averaged according to months for both hand-plucked and esophageal-fistula samples. The average in vitro cellulose digestibility of the pastures was highest in August (68.3%) as indicated by hand-plucked forages and lowest in September (59.5% and 55.8%) as

indicated by hand-plucked and esophageal-fistula forages, respectively.

Figure 4 shows the weekly in vitro cellulose digestibility curve of the pastures as obtained from hand-plucked forage samples and the weekly lactation curve, plotted as difference between actual and expected average daily milk yield of the ten selected cows. A strong negative relationship can be observed from the cellulose digestibility curve and the lactation curve up to the tenth week of the experimental period. However, after the tenth week the relationship appeared to be a positive one, that is, the difference between actual and expected lactation curve tended to decrease as the in vitro cellulose digestibility of the forages declined. The change in the relationship of the two curves after the tenth week might have resulted from the improvement in quality and quantity of the pastures due to an increased amount of rainfall (Table 5) in July and August. This could have increased the dry matter intake of the cows grazing, and subsequently increased their milk production.

It should be noted that the lactation curve shown in Figure 4 represents the average production of ten cows and the relationship observed with the cellulose digestibility curve of the pastures may not be exact if variations in milk production of individual cows were taken into account.

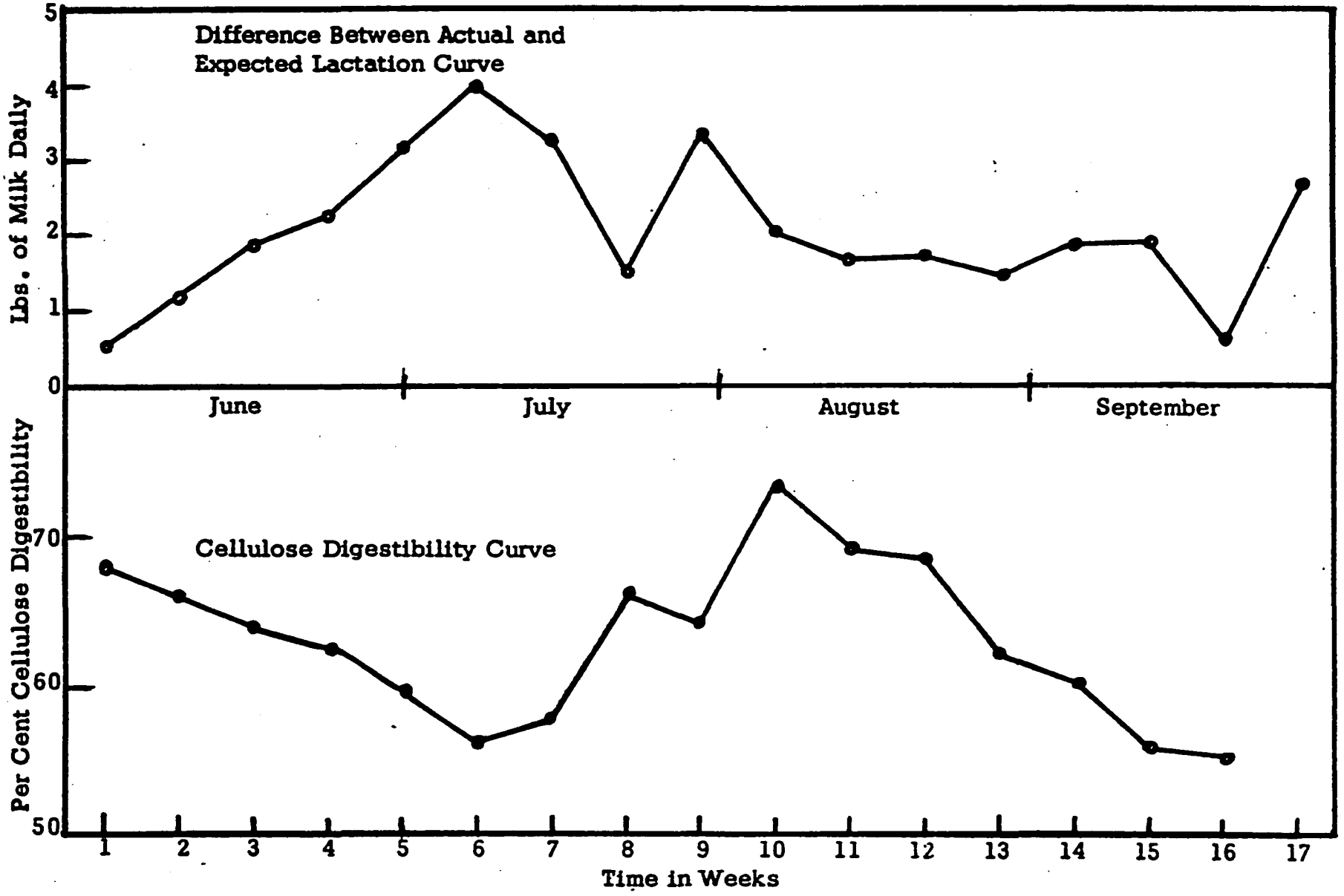

FIGURE 4. Lactation Curve (Weekly Difference between Actual and Expected Average Daily Milk Yield of Ten Cows) and Weekly In Vitro Cellulose Digestibility Curve of Pasture Using Hand-Plucked Samples

b. Volatile Fatty Acids Produced In Vitro

Volatile fatty acid (VFA) production of the pasture forages was determined from the liquid portion of the digest in the artificial rumen after a 48-hour incubation period. Acetic, propionic, butyric, and total VFA expressed as milligrams per gram of forage and the acetate/propionate ratio were determined.

Table 6 presents the weekly acetic acid and total VFA produced in vitro from the hand-plucked and esophageal-fistula pasture samples, averaged by months. It can be noted from the hand-plucked samples that both the average acetic acid and total VFA production increased in July and August and decreased in September. Similar trends in the average crude protein, ash, phosphorus, and potassium contents of the hand-plucked pasture samples can be noted in Table 4. The monthly VFA production trends appeared to be somewhat related to the in vitro cellulose digestibility trend for the hand-plucked pasture samples (Table 6). Similar trends in acetic acid and total VFA production can also be observed for the esophageal-fistula pasture samples in Table 6, although these trends did not appear to be related to the monthly in vitro cellulose digestibility and chemical composition of the esophageal-fistula pasture samples (Table 4).

The weekly in vitro production curves of acetic, propionic, and butyric acid, and total VFA for the hand-plucked pasture samples are shown in Figure 5. Weekly variations can be observed with acetic

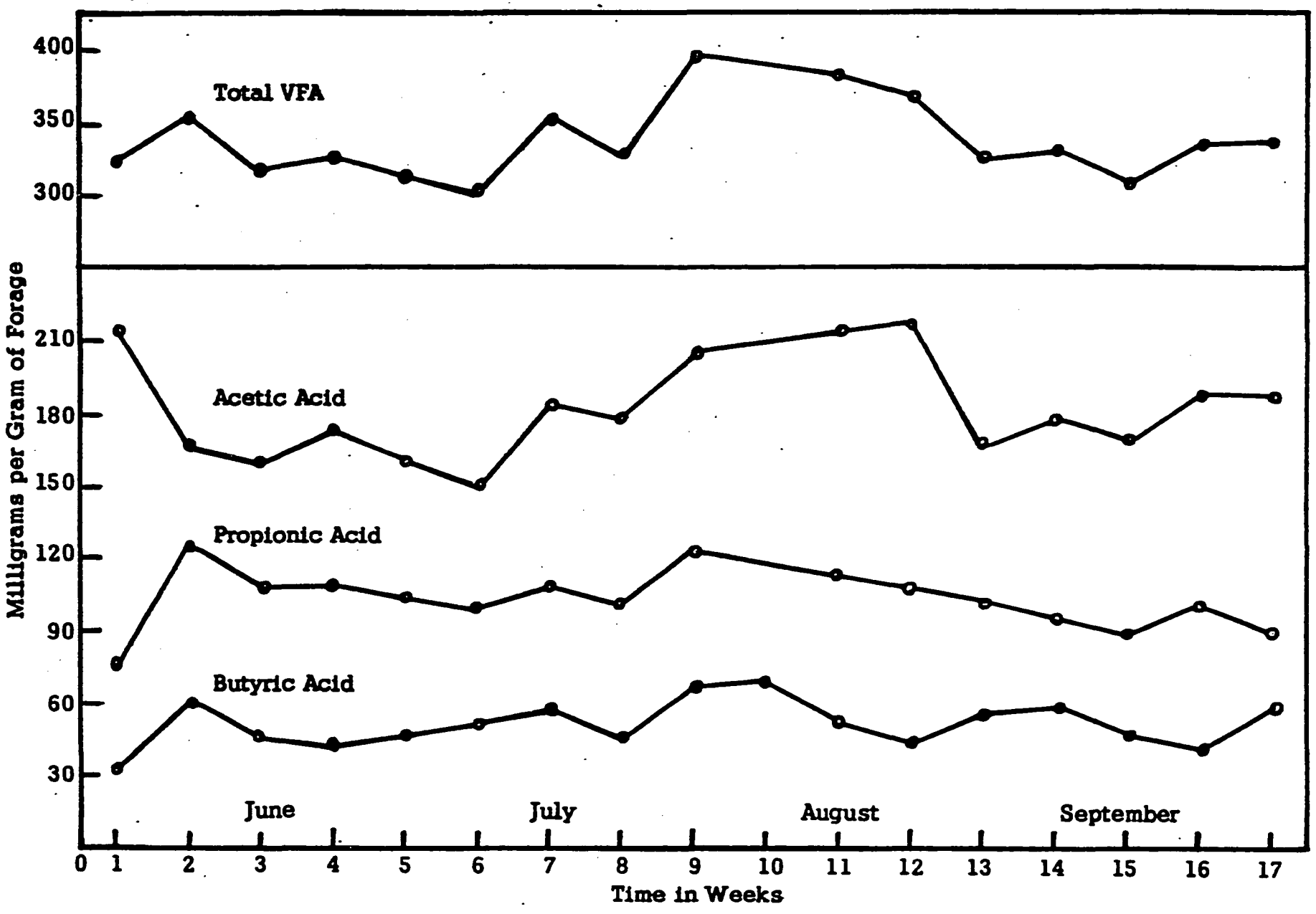

FIGURE 5. Weekly Volatile Fatty Acid Production <u>In Vitro</u> of Pastures Using Hand-Plucked Samples

acid and total VFA production. The greatest weekly total VFA production appears to occur in August. There seems to be little variation in the weekly production of propionic and butyric acids for the hand-plucked pasture samples. Figure 6 shows the weekly in vitro VFA production for the esophageal-fistula pasture samples. There appears to be less variation in weekly VFA production, but the highest trends in acetic acid and total VFA production seemed to occur in July and August, when the pastures appeared to be of better quality than during the other months (Table 4). These results would suggest that the in vitro production of VFA especially of acetic acid from the pasture forages could be an indication of the nutritional quality of pasture for dairy cattle.

C. Milk Production Data

1. Milk Yield

The daily milk production records of the ten cows selected from the station herd were used to calculate the expected milk yield, using the persistency factor, 0.9144, of Corley (23). This was done by taking the average daily production 10 days prior to the start of the experimental period and multiplying this by 0.9144. The product is the expected production on the first day of the experimental period. This product is again multiplied by the same factor to get the expected production 30 days after, and the same calculation is done for the

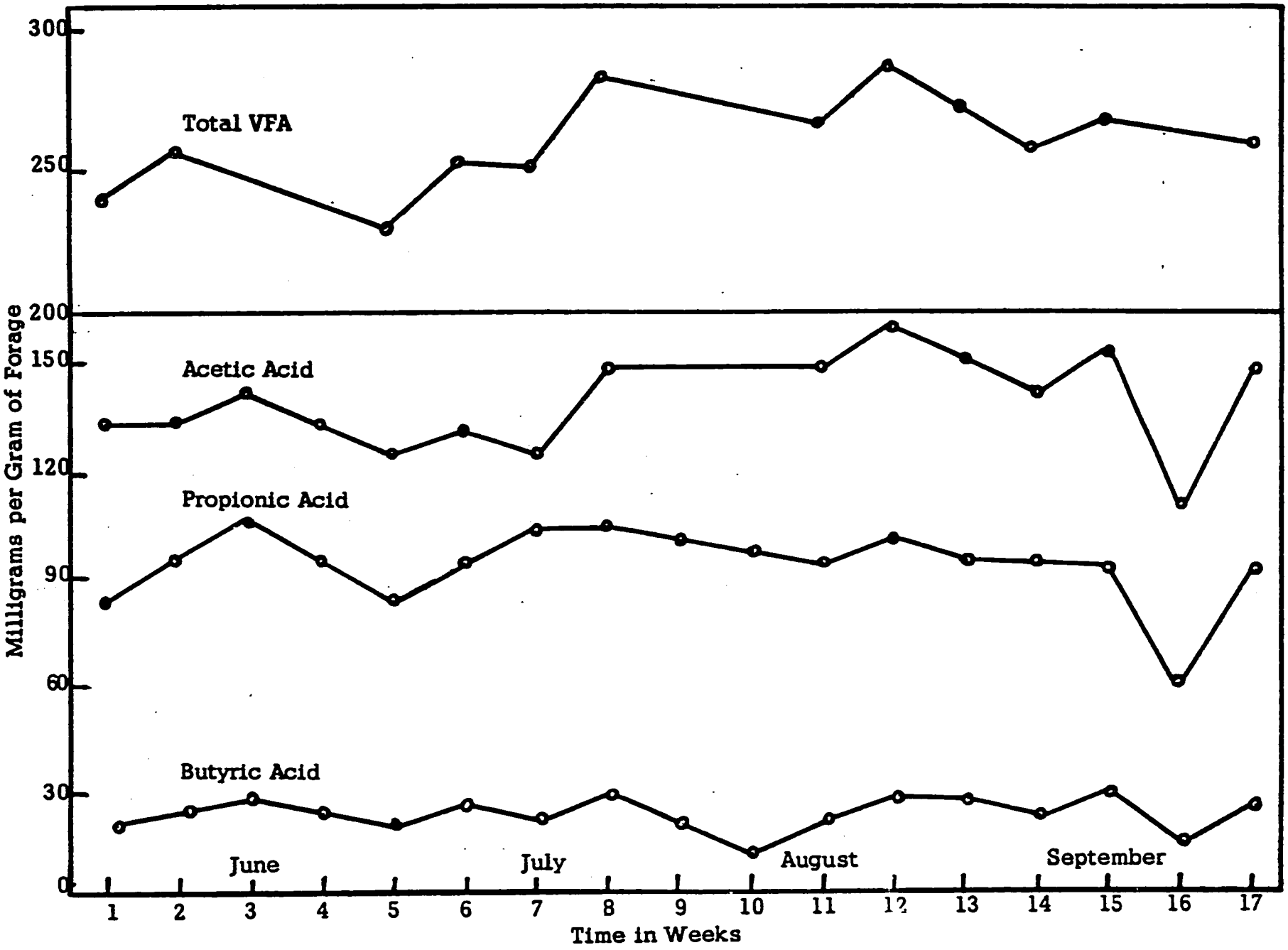

FIGURE 6. Weekly In Vitro Volatile Fatty Acid Production of Pastures Using Esophageal-Fistula Forage Samples

following 30 day intervals, so as to arrive at the expected lactation curve. The actual and expected average daily milk production of each of the ten selected cows are presented in Appendix Table 8a.

Bertrand (12) indicated that the persistency figure of Corley might be too high for Louisiana conditions since it was derived from lactation records of dairy herds in the northern section of the United States. It can be noted from the lactation curve in this study (Figure 4) that the average difference between actual and expected lactation of the ten cows was always below expected. It would certainly be more desirable to use a persistency factor based on lactation records of dairy herds in the South, since persistency is also known to be highly dependent on environmental factors (32).

2. Milk Fat and Solids-not-fat Production

The monthly milk fat and solids-not-fat production of ten cows are given in Appendix Table 9a. The increase in milk fat and solids-not-fat content of the cows' milk during the experimental period can be partially attributed to the decline in milk yield as lactation progresses. It is known that during the lactation period the percentages of fat, and more or less associated with this, the percentage of solids-not-fat in milk, tend to increase as lactation progresses and the amount of milk secreted decreases (39). Likewise the effect of hot weather and other environmental factors on milk fat and solids-not-fat production of dairy cows cannot be overlooked.

D. Relationship of Pasture and Climatic Factors Studied with Milk Production and Their Interrelationships with Each Other

1. Relationship of Factors between Hand-plucked and Esophageal-Fistula Pasture Samples

Since one of the objectives of this study was to evaluate the esophageal-fistula method of sampling pastures as compared to hand-plucking or simulated grazing, correlation coefficients were calculated on all chemical determinations for hand-plucked and esophageal-fistula forage samples from the Jersey cow. The correlation coefficients are given in Table 7. It should be noted that some of the significant correlation coefficients to be discussed accounted for less than 35 per cent of the associated variances as calculated by squaring the r values (r^2). It is important to keep this in mind in the discussion of significant relationships among variables.

The weekly *in vitro* cellulose digestibility of the hand-plucked pasture samples was significantly correlated ($r = 0.5771$, $P < .05$) with that of the esophageal-fistula samples, indicating also the reliability of hand-plucking or simulated grazing as a method of obtaining forage samples that are representative of forages actually grazed. The acetate/propionate ratios determined from the volatile fatty acids produced *in vitro* from hand-plucked and esophageal samples were significantly correlated ($r = 0.4891$, $P < .05$), although the correlations for the individual acids were nil. A wider ratio would be more desirable for dairy cows since acetic acid is the main precursor for

TABLE 7

Correlation between Hand-Plucked and Esophageal-Fistula Forage Samples Using 14 Different Criteria

X Variables	Correlation Coefficient
1. Cellulose digestibility in vitro	.5771*
2. Acetic acid production in vitro	.0423
3. Propionic acid production in vitro	.0281
4. Butyric acid production in vitro	-.3104
5. Total acid production in vitro	-.1673
6. Acetate : propionate ratio	.4891*
7. Crude protein content	.7511**
8. Crude fiber content	.5629*
9. Crude fat content	.2925
10. Nitrogen-free extract	.2390
11. Ash content	-.3039
12. Calcium content	.7602**
13. Phosphorus content	.3974
14. Potassium content	.6146**

* Significant ($P < .05$), $r_{15df} = .482$

**Highly significant ($P < .01$), $r_{15df} = .606$

the synthesis of milk fat. A highly significant correlation ($r = 0.7511$, $P < .01$) was obtained with the crude protein content of hand-plucked and esophageal-fistula samples and a significant correlation ($r = 0.5629$, $P < .05$) with the crude fiber content of the samples, indicating that hand-plucking could be fairly selective as compared to the cow grazing forage materials high in protein or low in fiber. A highly significant correlation ($r = 0.7602$, $P < .01$) was also obtained for the calcium content of the hand-plucked and esophageal-fistula forages, and a highly significant correlation ($r = .6146$, $P < .01$) was obtained for the potassium content of the forages. However, the ash contents of the two kinds of pasture samples were not significantly correlated ($r = -0.3039$) since the esophageal samples were contaminated with salivary minerals, especially phosphorus and sodium, and sometimes with soil material which the animal consumed.

The results discussed above would indicate that esophageal-fistula forages could be used in the artificial rumen to determine the in vitro cellulose digestibility of forages. Additional research work is needed to verify the results obtained.

2. Relationship of the Independent Variables with Each Other

a. Variables Determined from Hand-plucked Forages

Table 8 shows the correlation coefficients among the independent (X) variables and the dependent (Y) variable. Some combinations of the Xs and Y that showed no significant relationships were omitted from the

TABLE 8

Correlation Coefficients between the X-Variables[a] for Hand-Plucked Pasture Samples and the Y-Variable[b], Difference between Actual and Expected Milk Production

	X1	X2	X3	X4	X6	X7	X8	X9	X10	X13	X29	X31
X2	.375											
X3	.010	-.157										
X4	.170	.212	.446									
X5	.362	.716**	.618**	.318								
X6	.167	.629**	-.519*	-.151								
X7	.791**	.187	.338	.131	.153							
X8	-.561*	-.143	-.376	-.342	.270	-.364						
X9	.785**	.204	-.097	.213	.178	.908**	-.376					
X10	-.627**	-.079	-.056	.028	.105	-.790**	.440	-.722**				
X11	.363	.343	.285	-.061	.213	.408	-.369	.455	-.603*			
X12	.406	-.316	.174	-.062	-.605*	.452	-.486*	.193	-.474			
X13	.577*	.479	-.193	.112	.629**	.544*	.130	.640**	-.109			
X14	.785**	.459	-.208	.155	.579*	.472	-.048	.659**	-.198	.890**		
X29	-.065	-.203	.480	.531*	-.290	.175	-.319	.190	-.358	.243		
X30	.316	-.184	.036	.436	-.134	.259	-.177	.470	-.325	.287	.2214	
X31	-.065	-.203	-.321	-.343	.143	.173	.012	.169	-.118	.351	-.5841*	
Y	.1007	.0904	-.0713	-.1489	.0407	.0468	.0299	.0407	-.0127	.1088	-.2538**	.2365**

* Significant at $P < .05$; **Significant at $P < .01$

[a] X1 = cellulose digestibility in vitro, X2 = acetic acid production, X3 = propionic acid production, X4 = butyric acid production, X5 = total acid production, X6 = acetate/propionate ratio, X7 = crude protein content of pasture, X8 = crude fiber, X9 = crude fat, X10 = nitrogen-free extract, X11 = ash, X12 = calcium, X13 = phosphorus, X14 = potassium, X29 = total ambient air temperature, X30 = total rainfall, X31 = quality score of pasture.

[b] Y = actual minus expected daily milk yield, weekly average of 10 individual cows.

table. The correlation coefficients of the in vitro cellulose digestibility of hand-plucked forages (X1) with crude protein in forage (X7), crude fat in forage (X9), and potassium in forage (X14) were highly significant (r = .791, .785 and .785, respectively). In vitro cellulose digestibility was also significantly correlated (r = .577) with phosphorus in forage (X13), and showed a highly significant negative correlation (r = -.627) with NFE in forage (X10) and a significant negative correlation (r = -.561) with crude fiber in forage (X8). These results would indicate that forages high in protein, fat, and certain minerals, and also low in fiber and NFE would have high digestibilities of cellulose in vitro. It is interesting to note that the acetate/propionate ratio (X6) produced in vitro from hand-plucked forage showed a highly significant correlation (r = .629) with phosphorus in forage (X13), a significant correlation (r = .579) with potassium in forage (X14) and a significant negative correlation (r = -.605) with calcium in forage (X12). These would indicate that the minerals might be involved in the acetate/propionate ratio produced by rumen microorganisms from forages. Crude protein in forage (X7) showed a highly significant correlation (r = .908) with crude fat in forage (X9), a significant correlation (r = .544) with phosphorus in forage (X13) and a highly significant negative correlation (r = -.790)with NFE in forage (X10). This would indicate that forages high in protein might be high in crude fat and phosphorus contents and low in NFE. Phosphorus in forage (X13)

was highly correlated (r = .890) with potassium in forage (X14) indicating a close relationship between these two minerals. Total ambient air temperature (X29) showed a significantly negative correlation (r = -.5841) with pasture quality score (X31) indicating that as ambient air temperatures rise in the summer, the quality score of pastures would tend to decline. Total ambient air temperature (X29) showed a highly significant negative correlation (r = -.2538) with the difference between actual and expected milk yield (Y), and pasture quality score (X31) gave a highly significant correlation (r = .2365) with Y. However, r^2 values for these correlations with Y would indicate that they accounted for less than 10 per cent of the variance in Y.

b. Variables Determined from Esophageal-Fistula Forages

Table 9 shows the correlations between the independent (X) variables and the dependent (Y) variable. The in vitro cellulose digestibility of esophageal-fistula forages (X15) was significantly correlated (r = .509) with protein in esophageal-fistula forage (X21) and was highly correlated (r = .697) with calcium in forage (X26), indicating that esophageal-fistula forages high in protein and calcium would also have high cellulose digestibilities in vitro. The acetic acid produced from forages in vitro (X16) was highly correlated with propionic acid production (X17), butyric acid production (X18), and total VFA production (X19) with r = .835, .863 and .975, respectively; and acetic acid production was also significantly correlated (r = .579)

TABLE 9

Correlation Coefficients between the X-Variables[a] for Esophageal-Fistula Pasture Samples and the Y-Variable[b], Difference between Actual and Expected Milk Production

	X15	X16	X17	X18	X20	X21	X22	X25	X26	X29	X31
X16	-.039										
X17	.105	.835**									
X18	-.005	.863**	.853**								
X19	.011	.975**	.932**	.919**							
X20	-.308	.579*	.060	.284							
X21	.509*	.099	.347	.171	-.284						
X22	-.397	.279	.166	.245	.138	-.393					
X23	.337	-.230	-.149	-.204	-.277	.159	-.299				
X24	-.143	-.167	-.368	-.350	.213	-.596*	.103				
X25	-.292	-.155	-.322	-.240	.306	-.277	-.462				
X26	.697**	-.012	.164	.098	.256	.479	-.557*	.042			
X27	.092	-.251	-.371	-.197	.115	.205	-.554*	.464	.116	-.403	
X28	.368	.088	.141	-.062	-.007	.617**	-.343	.075	.113	.100	
X29	.066	-.156	.250	-.009	-.700**	.226	.267	-.540*	-.218	1.000	
X30	.081	.158	.133	-.044	.104	.118	.254	-.515*	-.151	.221	-.059
X31	.368	.284	.053	.120	.372	.030	-.157	-.012	.544*	-.584*	1.000
Y	.0406	.0856	-.0195	.0483	.1912*	.0351	-.0934	.1065	.1129	-.2538**	.2365**

* Significant ($P < .05$); **Highly significant ($P < .01$)

[a] X15 = cellulose digestibility, X16 = acetic acid production, X17 = propionic acid production, X18 = butyric acid production, X19 = total acid production, X20 = acetate/propionate ratio, X21 = crude protein, X22 = crude fiber, X23 = crude fat, X24 = NFE, X25 = ash, X26 = calcium, X27 = phosphorus, X28 = potassium, X29 = total ambient air temperature, X30 = total rainfall, X31 = pasture quality score.

[b] Y = actual minus expected daily milk yield, weekly average of ten individual cows.

with acetate/propionate ratio produced from the forages in vitro (X20). These relationships among the VFAs were to be expected since they were produced from the esophageal-fistula forages in similar proportions in the artificial rumen. Acetate/propionate ratio (X20) gave a highly significant negative correlation (r = -.700) with total ambient air temperature (X29) showing a negative relationship between air temperature and the acetate/propionate ratio produced from forages in vitro. The crude protein in forage (X21) showed a significant negative correlation (r = -.596) with NFE in forage (X24) and a highly significant correlation (r = .617) with potassium in forage (X28) indicating that esophageal-fistula forages high in protein would tend to have low NFE and high potassium content. The crude fiber in esophageal-fistula forage (X22) showed significant negative correlations (r = -.557 and -.554) with calcium in forage (X26) and phosphorus in forage (X27), respectively, indicating that esophageal-fistula forages high in crude fiber would tend to have low calcium and phosphorus contents. Pasture quality score (X31) was significantly correlated (r = .544) with calcium in forage (X26) indicating that the calcium content forages might be an indication of pasture quality. The significant correlations among Y, X29 and X31 have already been discussed in section (a) above.

3. Multiple Regression Analyses

Actual minus expected weekly average production on each of 10 cows was computed and used as the dependent variable, Y. The

regression of Y on the independent variables for hand-plucked and esophageal-fistula samples were analyzed separately. The results of the multiple regression analyses are presented in Appendix Tables 10a and 11a. None of the independent variables, Xs, showed a significant effect. Total volatile fatty acid production for hand-plucked and esophageal-fistula samples (X5 and X19) were removed because X5 was dependent on X2, X3, and X4 for the hand-plucked forages, and X19 was dependent on X16, X17, and X18 for the esophageal forages (Appendix Tables 10a and 11a). Appendix Table 10a gives an R^2 value of 0.10706, indicating that all fifteen independent variables were only accounting for 10.7 per cent of variation in Y. Appendix Table 11a gives a similar R^2 value of 0.10698. Each set of variables for the hand-plucked and esophageal forages together with total ambient air temperature (X29), total rainfall (X30) and pasture quality score (X31) accounted for about 10 per cent of the variation in actual minus expected milk production, weekly average (Y). Bertrand et al. (13) reported an $R = .31$ ($R^2 = .106$) for a multiple regression analysis of seven X variables (pasture quality score, mean daily temperature, per cent lignin, per cent crude protein, per cent NFE in herbage) on actual minus expected milk production, first day (Y). They found that pasture quality score was the only significant variable in predicting milk production among the variables studied. In this study pasture quality score failed

to predict milk production, even though it was highly correlated ($r = .2365$, $P < .01$) with Y, the difference between actual and expected milk production.

It can be noted in Appendix Table 8a that the average actual monthly production of those cows which were at the earlier stages of lactation (48-168 days or earlier) tended to be closer to or higher than the expected values. On the other hand, the actual production of those cows which were in the later stages of lactation (61-181 days or later) were always below the expected production. It can also be noted in Appendix Table 8a that the initial level of actual milk production of the cows varied from 23.8 to 46.2 pounds which might indicate differences in threshold effects, i.e., the cows on a higher initial level of production would probably respond better to changes in pasture quality than the cows on a lower level of production. Thus, such factors as stage of lactation and initial level of milk production might be involved in the way a cow's lactation would respond to external stimuli such as seasonal changes in pasture quality. Other factors including management conditions, weather, and previous status of the animals might also be involved. Finally, the measures of pasture quality used in this study would involve certain assumptions (e.g., the collection of representative forage samples) that could affect the accuracy of predicting the milk production of cows by these measures.

V. GENERAL DISCUSSION

The artificial rumen procedure that was developed for this particular study appeared to be an effective method of determining weekly changes in cellulose digestibility and volatile fatty acid production *in vitro* of pasture samples. By using the washed cell suspension technique described by Cheng *et al.* (22) it was possible to eliminate feed materials and other external factors from the cell inoculum. The *in vitro* procedure was unique in that it required only a bicarbonate buffer medium instead of a nutrient medium so that the rumen microorganisms depended entirely on the forage substrates to furnish them the necessary nutrients for optimum digestion of the forage substrates under study. Weekly digestibility trends of esophageal-fistula pasture samples were the same whether the source of rumen inoculum was from a rumen-fistulated cow grazing on pasture or from a cow being fed on drylot grain with alfalfa hay (Appendix Table 5a).

The chief advantage of the artificial rumen procedure used in this study appeared to be the speed with which digestibility determinations could be carried out in a series of fermentation flasks as compared with conventional digestion trials using live animals. This should be of importance primarily because of the large number of factors

involved in the efficiency with which rumen microorganisms could contribute to the nutrition of the host animal. Another advantage of the in vitro method would be the precision which could be exercised under the conditions in the laboratory which would be left to chance in experimental conditions using live animals. An important advantage of the artificial rumen method has to do with the economics involved since it does not involve the expense of feeding and care of animals in conventional digestion trials.

The weekly in vitro digestibility determinations carried out in this study (Tables 8 and 9) showed significant relationships with the weekly changes in the chemical composition of the pastures although they failed to support the pasture score system which was found of value as a simple method of pasture evaluation by Bertrand et al. (13). However, none of the criteria used in this study could predict milk production significantly. The failure of the pasture data to predict milk production might be due to the small number of samples (17 weekly observations) used in the study.

There appeared to be some relationships in monthly trends of the average ambient air temperature and total rainfall with those of the in vitro pasture cellulose digestibility, volatile fatty acid production, and the chemical composition of the pastures (Tables 5 and 6). In general, when there was ample rainfall and environmental temperatures were not too high during the summer season, the quality

of the pastures based on the chemical composition of hand-plucked pasture samples tended to be better. This was indicated by a rise in protein, fat, and mineral content of the pasture samples, and an increase in the in vitro cellulose digestibility and the volatile fatty acid production, especially that of acetic acid in the artificial rumen.

McCullough (61) at the Georgia station indicated that a one per cent change in digestibility of forage below the optimum digestibility value of 70 to 72 per cent would result in a two per cent change in milk persistency. When animal and plant variability was considered, differences of five per cent in digestibility would, on the average, produce highly significant changes in animal response. In this study changes of more than five per cent occurred in the in vitro cellulose digestibility of the pastures during some weeks (Figure 4).

The significance of VFAs as the main source of energy for ruminants has been discussed under the section on "Review of Literature." In this study VFAs were produced in vitro from the pastures in significant amounts (Table 6) with acetic acid constituting the major portion (50-55%). Weekly variations in total VFA and acetic acid production were observed (Figure 5). The ratio of acetate to propionate produced from the pastures in vitro also showed shifting trends which might be related to climate and changes in the chemical composition of the pastures (Tables 8 and 9). This has an important bearing on the efficiency with which forages are utilized by dairy cows

for milk production. Elliot and Loosli (36) reported that the efficiency with which digestible energy was converted to fat-corrected-milk was highly correlated with the relative proportion of propionic acid in the rumen VFA and with the ratio of acetate to propionate, as well as with the crude fiber content of the ration of dairy cows.

Certainly a more accurate prediction of the nutritive value of pastures for milk production would require a combination of the determination of digestibility and intake of energy with assessment of the amounts and relative proportions of the VFA end-products produced and absorbed in the rumen. The artificial rumen technique used in this study should be a very useful tool in the determination of available energy (cellulose digestibility) and VFA production from pastures by rumen microorganisms.

It is suggested that for further research the artificial rumen technique used in this study be applied to the determination of digestibility of permanent pastures for extended grazing periods in Louisiana, i.e., starting on early spring up to late fall. This will permit more forage samples to be collected, and more cows can be used to obtain milk production data. Thus an accurate prediction of milk yield could probably be made with the measures of pasture quality used in this study. The effect of stage of lactation on milk production response of cows to seasonal changes in pasture quality could be possibly eliminated.

IV. SUMMARY AND CONCLUSIONS

Research on the nutritive value of permanent pastures for dairy cattle in terms of chemical composition, pasture quality score, *in vitro* cellulose digestibility and volatile fatty acid production, and the resulting milk production of cows grazing on the pastures was conducted. The effect of ambient air temperature and rainfall on these measures of pasture quality, their interrelationships, and the reliability of each measure in predicting milk production were studied and analyzed.

Permanent pastures being grazed by the dairy herd at the Iberia Livestock Station, Jeanerette, Louisiana, were used to obtain weekly hand-plucked and esophageal-fistula forage samples for a 17-week experimental period (June 1, 1960 to September 28, 1960). Two esophageal-fistulated cows (Jersey and Holstein) were allowed to graze with the milking herd during sample collection. Ten cows selected from the herd, of different breeds and breed crosses, and at 40 to 120 days of their lactation, were used to obtain the milk production data. The factor 0.9144, of Corley was used to correct for the normal decline in lactation.

A washed cell suspension technique was used for the artificial rumen procedure. The forage substrate (0.2 gram) was incubated for

48 hours at 39.5 degrees C. in a buffer medium (pH 7.1) and furnished all the nutrients for microbial activity in the artificial rumen. Two rumen-fistulated steers that were being fed on drylot alfalfa hay and concentrate were used to provide rumen inoculum. Weekly in vitro cellulose digestibility and volatile fatty acid production of the pastures were determined.

Preliminary work with the artificial rumen showed that two-tenths gram of forage substrate gave optimum cellulose digestibility under the conditions of the experiment. A bicarbonate buffer me dium was found to be as satisfactory a medium for microbial digestion of forages as a nutrient medium. The pH values of 6.9 and 7.1 provided optimum microbial activity. Rate of digestion experiments using substrates of hand-plucked and esophageal forages showed maximum cellulose digestion of the forages at 40 to 48 hours of incubation. Two different sources of rumen inoculum--one a rumen-fistulated steer grazing on permanent pasture, and a rumen-fistulated steer fed grain with alfalfa hay on drylot--gave similar weekly cellulose digestibility trends of the pastures. Based on these studies, it was decided not to use the esophageal-fistula samples from the Holstein cow for the forage evaluation experiments.

In general, the pastures were higher in crude protein, crude fat, phosphorus and potassium, and lower in crude fiber and NFE when there was ample rainfall and ambient air temperatures during the

summer were somewhat lower. The esophageal-fistula forages were much lower in dry matter, higher in crude fiber, lower in crude fat and NFE than the hand-plucked forages.

The average *in vitro* cellulose digestibility of the hand-plucked pasture samples was highest in August (68.3%) and lowest in September (59.5%), whereas that of the esophageal-fistula pasture samples did not change much in June, July, and August (63.6, 62.0 and 62.4%, respectively) but dropped in September (55.8%) probably due to the decreased quality of the pastures.

The total volatile fatty acid (VFA) production *in vitro* was highest in August for both the hand-plucked and esophageal samples (349.9 and 248.5 mg. per gram forage) and was lowest in September for hand-plucked forages and in June for esophageal-fistula forages. Acetic acid (50-55% of total VFA) followed similar trends as the total VFA.

In general, the *in vitro* cellulose digestibility and total volatile fatty acid production of the hand-plucked pasture samples were greater when the samples were higher in crude protein, crude fat, phosphorus, and potassium, and lower in NFE.

Correlation analysis between weekly-collected,hand-plucked and esophageal-fistula forage samples showed significant relationships for crude protein, crude fiber, potassium, *in vitro* cellulose digestibility, and the acetate/propionate ratio produced from the forages *in vitro*. The esophageal-fistula forages were constantly lower in crude fat and

NFE, and higher in ash, phosphorus, and sodium contents than the corresponding hand-plucked forages.

In general, the weekly trends in the in vitro cellulose digestibility of the pastures were significantly correlated with the weekly changes in crude protein, crude fat, calcium, phosphorus, and potassium contents of the pastures, and showed significantly negative correlations with the changes in crude fiber and NFE of the pastures.

The average weekly quality score of the pastures was not related to the weekly trends in chemical composition and in vitro cellulose digestibility of the pastures, except for calcium content of the pastures. However, a significantly negative relationship existed between pasture quality score and total ambient air temperature (maximum plus minimum).

Multiple regression analyses indicated a failure of all measures (Xs) of pasture quality and climate to significantly predict milk production (data obtained from ten cows). R^2 values indicated less than ten per cent of variation in Y (actual minus expected milk yield) accounted for by the Xs.

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VIII. APPENDIX

INSTRUCTION SHEET FOR PASTURE SCORE SYSTEM

1. Type of Pasture

State botanical composition of pasture, e.g. Bermuda grass, white clover, and Dallis grass. Give the approximate percentages of each of the grasses and clovers in the pasture. State time on pasture.

2. Quantity of Pasture

The fact as to whether or not the cows can get good fill in a reasonable grazing period is referred to the scores for surplus, adequate and deficient.

21 - 30 = surplus

11 - 20 = adequate

0 - 10 = deficient

3. Quality Scores

This is more or less explanatory. Give particular attention to coarseness, stage of maturity and the succulence of the pastures.

31 - 40 = excellent - excellent growth and succulence

21 - 30 = good - young and succulent growth

11 - 20 = fair - some feed coarse and mature

0 - 10 = poor - mostly coarse and mature. No succulence.

4. Weather Conditions

Record the amount of rainfall, if any, each day. State whether clear, partly cloudy or very cloudy. Record maximum and minimum ambient temperatures.

5. Give description of the supplemental forage feeding program.

TABLE 1a

Composition of Nutrient Solution (22)

Chemicals	Gram per 2 liters
Na_2HPO_4	0.631
KH_2PO_4	0.303
$NaHCO_3$	5.250
KCl	0.750
NaCl	0.750
$MgSO_4$	0.225
$CaCl_2$	0.075
$FeSO_4 \cdot 7H_2O$	0.015
$MnSO_4$	0.008
$ZnSO_4 \cdot 7H_2O$	0.008
$CuSO_4 \cdot 5H_2O$	0.004
$CoCl_2$	0.002
Urea	2.000

TABLE 2a

Analysis of Variance for the In Vitro Cellulose Digestibility of Forage as Affected by Weight of Substrate and Kind of Medium

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F Ratio
Replicates	1	5.04	5.04	1.18
Treatments	11	1569.70	142.70	33.50
A (Kind of medium)	3	1041.35	347.12	81.48**
B (Wt. of Substrate)	2	270.04	135.02	31.69**
AB	6	258.31	43.05	10.11**
Error	11	46.83	4.26	
Total	23	1621.57		

**Highly significant ($P < .01$)

TABLE 3a

Analysis of Variance for the In Vitro Cellulose Digestibilities of Hand-Plucked and Esophageal-Fistula Forages as Affected by Kind of Medium and Cell Concentration

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F Ratio
Replicates	1	4.95	4.95	less than 1
Treatments	11	11705.31	1064.12	81.35
A (Tmt. medium)	3	214.52	71.51	5.47*
B (Kind of Forage)	2	11399.09	5699.55	435.75**
AB	6	91.70	15.28	1.17
Error	11	143.86	13.08	
Total	23	11854.12		

* Highly significant ($P < .01$)

**Significant ($P < .05$)

TABLE 4a

Analysis of Variance for the In Vitro Cellulose Digestibility of Hand-Plucked and Esophageal-Fistula Forages as Affected by pH of Medium

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Ratio
Forages	3	51.50	17.17	1.87
Treatments	3	185.89	61.96	6.76*
Error	9	82.55	9.17	
Total	15	319.94		

* Significant ($P < .05$)

TABLE 5a

In Vitro Cellulose Digestibility of Esophageal-Fistula and Hand-Plucked Forage Samples Using Two Different Sources of Rumen Inoculum

Forage No.	Date of Collection	Esophageal (Jersey)		Hand-Plucked	
		Pasture[a] Inoculum	Alfalfa[b] Hay Inoculum	Pasture Inoculum	Alfalfa Hay Inoculum
		% Cellulose Digestibility[c]			
1	5/31/60	63.5	65.1	50.3	65.1
2	6/8	62.4	65.9	45.6	65.9
3	6/17	56.3	63.4	42.7	63.4
4	6/24	38.0	29.8	42.1	63.0
5	7/1	60.3	62.2	43.4	62.2
6	8/8	55.0	64.1	41.7	64.1
7	7/15	53.6	55.5	40.4	55.5
8	7/22	66.8	68.3	-	68.3
9	7/29	55.8	62.6	51.6	62.6
10	8/6	62.5	63.5	61.7	63.5
11	8/13	60.3	67.5	55.8	67.5
12	8/19	52.6	51.4	52.5	59.5
13	8/26	49.4	56.2		
14	9/3	49.4	56.5		
15	9/13	47.5	56.9		
16	9/17	49.4	55.8		
17	9/28/60	53.0	58.1		
Alfalfa hay standard		44.7	58.2		

[a] Rumen inoculum from cow grazing on summer pasture
[b] Rumen inoculum from cow being fed alfalfa hay plus concentrate on drylot
[c] Each value is an average of two in vitro digestion trials

TABLE 6a

Correlation Analysis on In Vitro Cellulose Digestibilities of Esophageal-Fistula Forages Using Two Sources of Rumen Inoculum

$n = 16,$	$\bar{x}_1$ [a] $= 56.1,$	$\bar{x}_2$ [b] $= 61.3$
$Sx_1^2 = 523.26,$	$Sx_2^2 = 288.78,$	$Sx_1x_2 = 344.94$
$r = 0.8874^{**},$	$r^2 = .7875$	

[a] Mean per cent cellulose digestibility using rumen inoculum from steer on pasture.

[b] Mean per cent cellulose digestibility using rumen inoculum from steer on drylot.

** Highly significant ($P < .01$)

TABLE 7a

Correlation Analysis on In Vitro Cellulose Digestibilities of Hand-Plucked Forages Using Two Sources of Rumen Inoculum

$n = 11,$	$\bar{x}_1$ [a] $= 47.9,$	$\bar{x}_2$ [b] $= 62.9$
$Sx_1^2 = 474.30,$	$Sx_2^2 = 104.64,$	$Sx_1x_2 = 73.80$
$r = .3313$	$r^2 = .1089$	

[a] Mean per cent cellulose digestibility using rumen inoculum from steer on pasture.

[b] Mean per cent cellulose digestibility using rumen inoculum from steer on drylot.

TABLE 8a

Actual and Expected Milk Yield of Ten Cows During the Experimental Period
(June 1, 1960 to September 28, 1960)

Cow No.	Stage of Lactation	June		July		August		September	
		Average Daily Milk Production							
		Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected
		(lb.)							
H-215	48-168	25.2	29.1	22.4	26.5	22.8	24.4	21.4	22.4
H-213	41-161	33.9	34.5	30.6	31.4	30.8	28.9	30.2	26.6
J-342	61-181	30.3	31.1	25.5	28.5	21.1	26.1	19.0	24.0
J-310	106-226	23.8	26.3	17.4	24.0	16.9	22.0	15.4	20.2
S-63	65-185	38.2	41.6	35.8	37.9	31.8	34.8	31.1	31.9
SX-250	115-235	35.2	37.0	31.9	33.7	29.6	31.0	26.6	28.4
SX-17	61-181	34.5	34.5	28.5	31.4	26.8	28.9	23.1	26.6
SX-25	42-162	46.2	44.1	41.4	40.1	38.6	36.8	19.7	33.8
X-451	44-164	33.4	34.5	28.5	31.4	25.6	28.9	28.7	26.6
X-454	41-161	25.3	26.3	25.3	24.0	25.1	22.0	23.9	20.2
Mean		32.6	33.9	28.7	30.9	26.9	28.4	24.9	26.1

TABLE 9a

Milk Fat and Solids-not-fat Content of Each Cow's Milk During the Experimental Period

Cow No.	June		July		August		September	
	Fat[a]	SNF[b]	Fat	SNF	Fat	SNF	Fat	SNF
	%							
X-451	3.09	8.45	3.34	8.55	3.92	8.84	3.28	8.66
X-454	4.08	8.41	2.35	8.67	2.27	8.80	4.26	8.85
SX-25	3.70	8.31	2.50	8.44	2.84	8.46	3.98	8.45
SX-250	3.47	8.54	3.83	8.36	4.41	8.78	4.07	8.71
S-63	2.28	7.16	2.92	7.77	3.29	7.82	2.88	7.61
SX-17	4.63	8.62	4.75	8.64	4.34	8.70	5.53	8.74
J-310	4.59	9.02	5.42	8.66	4.87	8.61	5.13	8.46
J-342	3.61	8.31	4.30	7.91	4.05	8.31	3.98	8.39
H-215	2.91	8.11	3.41	8.11	3.18	8.36	3.35	8.19
H-213	3.53	8.34	3.39	7.95	4.20	8.38	3.57	8.32
Mean	3.59	8.33	3.62	8.31	3.75	8.51	4.00	8.44

a/ Milk fat

b/ Solids-not-fat

TABLE 10a

Multiple Regression Analysis on Milk Yield Using Pasture Data Obtained from Hand-Plucked Samples

	X Variables	Regression coefficient[a]	F ratio[b]
X1	(Cellulose digestibility in vitro)	.532	.924
X2	(Acetic acid production in vitro)	.020	.465
X3	(propionic acid production in vitro)	- .042	.282
X4	(Butyric acid production in vitro)	- .206	.980
X6	(Acetate :propionate ratio)	- 8.422	1.180
X7	(Crude protein in forage)	4.325	1.054
X8	(Crude fiber in forage)	.435	.492
X9	(Crude fat in forage)	- 9.431	.798
X10	(NFE in forage)	1.779	.932
X11	(Ash in forage)	- .008	.0001
X12	(Calcium in forage)	-34.436	.887
X13	(Phosphorus in forage)	-70.826	1.483
X14	(Potassium in forage)	4.679	2.236
X29	(Total ambient temperature)	.082	.099
X30	(Weekly total rainfall)	.805	.667

$R^2 = .10706$

[a] Regression coefficient of Y (actual minus expected milk, weekly average of each of 10 cows) on the Xs.

[b] Degrees of freedom = 1 and 153; F .05 = 3.90.

TABLE 11a

Multiple Regression Analysis on Milk Yield Using Data Obtained from Esophageal-Fistula Pasture Samples

	X Variables	Regression coefficient[a]	F ratio[b]
X15	(Cellulose digestibility in vitro)	- .004	.0001
X16	(Acetic acid production in vitro)	-.070	.0179
X17	(Propionic acid production in vitro)	.057	.0094
X18	(Butyric acid production in vitro)	.106	.0249
X20	(Acetate : propionate ratio)	3.319	.0254
X21	(Crude protein content)	- .014	.0000
X22	(Crude fiber content)	.057	.0004
X23	(Crude fat content)	.829	.1549
X24	(Nitrogen-free-extract)	- .229	.0047
X25	(Ash content)	- .067	.0004
X26	(Calcium content)	-1.110	.0155
X27	(Phosphorus content)	-9.467	.6607
X28	(Potassium content)	1.652	.1847
X29	(Total ambient temperature)	- .194	.6883
X30	(Total rainfall)	- .065	.0163
X31	(Pasture quality score)	.085	.0322

$R^2 = .10698$

[a] Regression coefficient of Y, the adjusted weekly milk yield of each of 10 cows on X

[b] Degrees of freedom = 1 and 153; F .05 = 3.90.

AUTOBIOGRAPHY

Antonio Lino Ordoveza was born in Manila, Philippines on March 17, 1935. He received his elementary and high school education at the Ateneo de Manila, finishing in 1951. He did his undergraduate work at the College of Agriculture of the University of the Philippines and received the B. S. degree in Agriculture in 1955.

He came to the United States in 1956 and received the M. S. degree in Animal Husbandry at Kansas State University, Manhattan, in 1958.

He then transferred in the fall of 1958 to North Carolina State College, Raleigh, to continue graduate work in animal nutrition. He met for the first time, in Raleigh, his wife, who is a native of the Philippines and comes from his neighboring province.

In the fall of 1959 he came to Louisiana State University to continue work toward the degree of Doctor of Philosophy in Dairy Nutrition.

EXAMINATION AND THESIS REPORT

Candidate: Antonio Lino Ordoveza

Major Field: Dairy Nutrition

Title of Thesis: Nutritional Evaluation of Pastures for Dairy Cattle in Louisiana Using In Vitro Methods

Approved:

L. L. Ruseff
Major Professor and Chairman

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Date of Examination:

December 13, 1962